UNIVERSITY
OF MICHIGAN
AUG 3 1955
SCIENCE
LIBRARY

HEREDITAS

GENETISKT ARKIV

UTGIVET AV MENDELSKA SÄLLSKAPET I LUND

REDAKTÖR: ARNE MÜNTZING

BAND 41: 1—2 1955

BERLINGSKA BOKTRYCKERIET, LUND

HEREDITAS

a periodical devoted to the publication of original research in genetics and cytology, is published by the MENDELIAN SOCIETY of Lund, Sweden. All contributions are written in English, German or French, illustrated, when necessary, with adequate text figures and plates. The current volume (41) will contain about 500 pages, issued in four numbers.

Subscriptions should be sent to the Editor. Price *Thirty-five* (*35*) *Swedish Kronor per volume.* Postage free.

Back volumes. — The price of Vols. I—XII and XIX—XX is 15 Sw. Kr. each, of Vols. XXVI—XXXIII and XXXVII, 25 Sw. Kr. each, and of Vols. XXXIX—XL 35 Sw. Kr. each. Postage free.

(Vols. XIII, XIV, XV, XVI, XVII, XVIII, XXI, XXII, XXIII, XXIV, XXV, XXXIV, XXXV, XXXVI, XXXVIII, and Suppl. Vol. 1949 are entirely out of print.)

ARNE MÜNTZING
Editor of »Hereditas»
Institute of Genetics, LUND, SWEDEN.

REDAKTIONSKOMMITTÉ

PROFESSOR, FIL. DR. *J. RASMUSSON*
PROFESSOR, FIL. DR. *HERIBERT NILSSON*
PROFESSOR, FIL. DR. *GERT BONNIER*
PROFESSOR, MED. DR. *ERIK ESSEN-MÖLLER*

Science

HEREDITAS

GENETISKT ARKIV

UTGIVET AV MENDELSKA SÄLLSKAPET I LUND

REDAKTÖR: ARNE MÜNTZING

BAND 41 1955

BERLINGSKA BOKTRYCKERIET LUND

CYTOGENETIC STUDIES ON RELATIONSHIPS BETWEEN ANNUAL SPECIES OF LAMIUM

By *PETER BERNSTRÖM*

HILLESHÖG, LANDSKRONA, SWEDEN

INTRODUCTION

IN earlier studies attention has been drawn to investigations that have been carried out on the relationships between some species of *Lamium* occurring in northern Europe; only preliminary results from these have so far been published (BERNSTRÖM, 1949, 1952, 1953 a and b). The original aim of the investigations was to ascertain whether either of the tetraploid species *Lamium hybridum* VILL. and *L. intermedium* FR. or both had resulted from a doubling of the chromosome number in connection with hybridization between the diploid species *L. purpureum* L. and *L. amplexicaule* L. The two tetraploids have appearances which are, in their respective ways, intermediate between those of the diploid species, and the systematic position of the tetraploid species in relation to that of the diploid species has long constituted a stumbling block for taxonomists; the differences in the views held as to the systematic position of the respective species in different floras are sufficiently eloquent in this connection. The historical aspect of the genetic side of the question has been discussed in an earlier study, in which, further, the species referred to have been presented in detail (BERNSTRÖM, 1952, 1953 b).

In a previous study an account has been given of the method of increasing the crossability between the four above-mentioned species by doubling the chromosome numbers of the diploid species (BERNSTRÖM, 1953 a). Before this it had only been possible to produce hybrids between *L. amplexicaule* and *L. hybridum*. With the procedure mentioned above it was possible to produce hybrids of several combinations of species, which enabled the analysis of the relationships between the species of which an account is given below. For further details concerning the compatibility and the incompatibility between the different species the reader is referred to the study in question.

In investigations of the phylogeny of the species several questions of rather more general scope have been encountered and made the object of closer study, also because they belong to the studies on the relationships between the species, and it has been necessary to deal with them in order to throw light upon those studies. Some questions of intraspecific nature have been dealt with in earlier works, such as the general occurrence of interlinear partial sterility in *L. amplexicaule,* the cytological instability of *L. intermedium*, the common occurrence of heterosis in hybrids in *L. amplexicaule* and *L. purpureum*, fertility and aneuploidy and the manifestations of this in autotetraploids of these two species, *etc.* (BERNSTRÖM, 1950 a and b, 1952, 1953 b, 1954). Other questions of interspecific character will be dealt with below.

During the period 1943—1950 the investigations were carried out at the Institute of Genetics, University of Lund, and 1951—1954 at the Hilleshög Sugar Beet Breeding Institute, Landskrona.

MATERIAL AND METHODS

A detailed account of the methods, *etc.*, used in the investigations, has been given in a couple of earlier studies, to which the reader is herewith referred (BERNSTRÖM, 1952, 1954). Especially from the cytological viewpoint the species studied have not constituted a suitable material for experiment, as the chromosomes are small, the pollen mother cells are few in each anther, there are only 4 anthers to each flower, and the flowering is so prolonged that it is difficult to find the few buds which at the same time undergo reduction divisions. The buds are very small at this stage, and it is necessary to cut off the branches in order to find them. Further, it has as a rule proved very hard to get good fixations of reduction divisions. Thus behind the few or single slides of each hybrid combination, *etc.* that it has been possible to study more closely there generally lie extensive and repeated fixations of, in the majority of cases, several line combinations, from which the best slides, or those that it has been possible to use at all, have been chosen. Owing to these and other difficulties, which will emerge from the following, the investigation has unavoidably come to suffer from certain defects.

Also the material of the pure species used for the experiments has been dealt with earlier (BERNSTRÖM, 1952, 1953 b, 1954). The only exception is the line of *L. bifidum* CYR. subsp. *balcanicum* VEL. used in species hybridizations. *L. bifidum* is an annual species occurring in Italy and on the Balkan peninsula, whose subsp. *balcanicum*, which occurs

only on the Balkan peninsula, shows, rather than the main form, a close agreement, in its general appearance, with the annual species occurring in northern Europe, and may therefore be presumed to be closely related to these. The species proper has white flowers, while the flowers of the subspecies are red. Both have $2n=18$. In spite of repeated attempts, it was not possible to obtain seed of *L. bifidum* subsp. *balcanicum* until 1949, when a sample was obtained from Sophia. A sample of *L. bifidum* was obtained from Catania.

Both *L. bifidum* and its subspecies seem to be winter annuals and/or have a tendency to short-day flowering. It has proved difficult to get them to flower in greenhouse cultures simultaneously with the other species with which it had been intended to cross-pollinate them, and to get them to flower chasmogamically at all; they are dimorphous, as is also *L. amplexicaule*. For this reason it has only been possible, in spite of repeated attempts, to cross-pollinate to any extent worth mentioning *L. bifidum* subsp. *balcanicum* with *L. purpureum,* and *vice versa*. At the time for *L. bifidum*'s flowering *L. amplexicaule* did not flower, or had only cleistogamic flowers. Attempts to cultivate *L. bifidum* have been carried out chiefly with subsp. *balcanicum*. As will be shown in greater detail below, it was possible to produce hybrids between *L. purpureum* and *L. bifidum* subsp. *balcanicum*, and these have been of value for the analysis of the relationships between the species.

EXPERIMENTAL

I. Tetraploid F_1 hybrids

A. L. intermedium×tetraploid L. purpureum

a. *Cytology*

The chromosome numbers in hybrids of two different line combinations have been checked and found to be $2n=\pm 36$.

It has been possible to study two slides with meiotic divisions, both in I M, of $i47\times p30$. As in the case of the autotetraploids (BERNSTRÖM, 1954) and also of other tetraploid hybrids (*cf.* below), it proved difficult to distinguish definitely between trivalents and quadrivalents, where the latter occurred, and between bivalents and trivalents; the chromosomes were too small and contracted for the configurations to be sufficiently differentiated for reliable identification. Although it was, certainly, possible to classify many of them, it was unusual for a whole cell to be

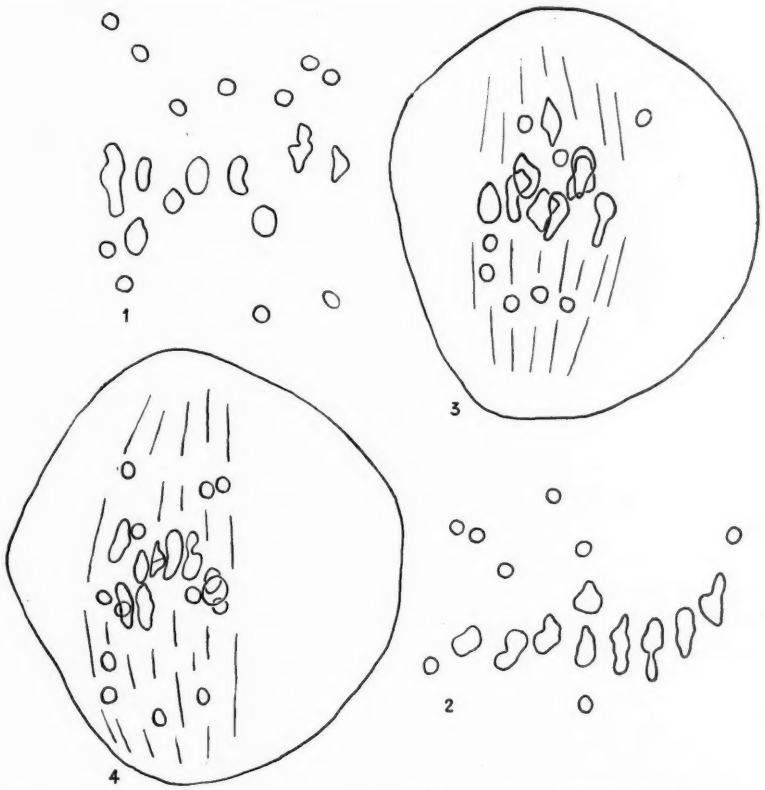
TABLE 1. *Chromosome pairing at I M in hybrids between L. intermedium and tetraploid L. purpureum.*

Per cell / Slide No.		6	7	8	9	10	11	12	8	9	10	11	Number of cells
	I's												
	Conf's	9	9	9	9	9	9	9	10	10	10	10	
1 ($2n=36$)		—	—	5	34	9	6	1	3	1	1	—	60
2 ($2n=35$)		2	6	17	5	1	—	—	1	1	—	2	35

definitely analysable in this way, and it was therefore necessary to separate the chromosome associations into univalents+configurations. The difficulties were not, however, so great that one could not clearly observe that quadrivalents were sparse, bivalents much commoner, but trivalents and univalents predominant. Nothing that could be suspected of being a pentavalent was observed. In Table 1 will be found the frequencies of cells with observed number of univalents+configurations per cell.

The observation that trivalents were by far the commonest configurations is supported by the fact that in the majority of cells there are 9 configurations, never fewer, and only in a few cells 10, at the same time as most cells also had 9 univalents. This, however, is the case in only one of the two buds studied, and the majority of cells had for the other 8 univalents. Such a displacement may be expected if the plant was hypotetraploid with $2n=35$; an attempt to determine the chromosome number in somatic divisions in the bud gave the results ± 35, ± 32, 35. The last metaphase plate was clear. Thus also the second bud shows that 9 I+9 III is the commonest mode of pairing in this hybrid. This would then imply that *L. intermedium* must be an allotetraploid, and that the one of its genomes whose chromosomes, as a rule, form only bivalents (BERNSTRÖM, 1953 b) must be homologous with that of *L. purpureum*.

The occurrence of cells with fewer univalents than 9 or 8 simultaneously with 9 configurations may perhaps in a few cases imply that the remaining univalent or univalents are hidden; but for the majority of cases this is scarcely likely, as the analysis was carefully made. The combination thus probably implies the occurrence of a quadrivalent; these have, moreover, been found in definitely two and in all likelihood several cells with at least 9 univalents. The presence of quadrivalents may signify either that one or more chromosomes of the odd genome (*i. e.*, the univalent-forming genome) in the hybrids are partially homo-



Figs. 1—4. I M in *L. intermedium*×tetraploid *L. purpureum*. PMC's, depicted from the same plant, with $2n=36$. — Fig. 1, most probably 1 IV+5 III+3 II+11 I; Figs. 2 and 3, cells having 9 configurations+8 I. The IV's which must occur are not discernible. Fig. 4, probably 6 III+3 II+12 I. The chromosomes separately drawn in Figs. 1 and 2. — ×2500.

logous with the *L. purpureum* chromosomes, in which case multivalents may occur also in pure *L. intermedium* (as is, moreover, actually the case; BERNSTRÖM, 1953 b), or that structural differences such as translocations or duplications occur between the *L. purpureum* genome and the genome homologous therewith or, of course, a combination of the two possibilities. The cells with 9 configurations+8 (7) I indicate the first alternative, that with partial homology.

Fig. 1 shows the chromosomes from a cell of slide No. 1, the chro-

mosomes being separately drawn with their respective levels retained; they very probably formed 1 IV+5 III+3 II, besides 11 I. It is seldom, however, that the quadrivalent is as clear as this; Figs. 2 and 3 show two different cells from slide No. 1, each having only 8 I, and they should thus have one quadrivalent each. It is difficult, though, to point these out. Fig. 4, finally, has 12 I, probably no quadrivalent, but 3 II+6 III.

The occurrence of 10 configurations in one PMC is explained in the same way as that of a quadrivalent; in place of this, however, two bivalents have been formed.

b. *Fertility and morphology*

Owing to the difficulty in producing hybrids between *L. intermedium* and tetraploid *L. purpureum* (*cf.* BERNSTRÖM, 1953 a, Table 1), only 5 hybrid plants were studied. Two derived from the combination $i22 \times p7$ and were cultivated during the autumn generation 1944, while three came from the combination $i47 \times p30$. These were cultivated in the autumn of 1945.

Four of the five hybrid plants were very similar in appearance, while the fifth was clearly divergent. The four plants were a good deal more weakly developed than the parents, with, *inter alia*, smaller leaves, which had a characteristic bowl-shaped appearance. The plants also showed other signs of lesser vitality and disharmonious development. Fig. 5 shows a hybrid plant between its parents; Fig. 6 shows the inflorescence of the hybrid.

The morphology of the hybrids was in other respects rather intermediary, habitually as well as regarding the shape of the leaves, calyces, flowers, *etc.* At the beginning of the flowering the flowers were almost as large as those of tetraploid *L. purpureum*, but they then diminished successively in size and finally remained entirely closed, with a length of only 6—7 mm. The anthers, too, gradually stopped dehiscing. Only one seed was obtained from all the plants. The pollen grains varied as to size and stainability, as might have been expected with a knowledge of the chromosome-pairing; only about 10 per cent of the grains were stained.

The plant diverging from the other hybrids was large and perfectly harmoniously developed. The leaves were quite as big as those of the parents; the plant had, moreover, a longer flowering period with open flowers than two sister-plants. There could be no doubt as to its nature of a species hybrid.

Fig. 5. To the left *L. intermedium* (*i*22), to the right tetraploid *L. purpureum* (*p*7), in the centre their F_1 hybrid.

Fig. 6. Inflorescence of F_1 hybrid between *L. intermedium* (*i*22) and tetraploid *L. purpureum* (*p*7). The plant is depicted in Fig. 5.

Two causes of the occurrence of this divergent plant are conceivable. One is that it was the aneuploid whose occurrence was revealed by the cytological examination. For a plant in a hybrid combination which is characterized by a reduced vitality and a distinct physiological unbalance it is not unreasonable to expect that the loss of a chromosome may entail a genetic improvement inasmuch as the chromosome in question has carried a gene, or genes, not harmonizing with the genomes of the opposite parent. The other conceivable cause is that the vitality of the hybrids in this combination of species will depend on what line of *L. purpureum* has been used. For in the combination *L. hybridum*× tetraploid *L. purpureum* the development of the hybrids is in a high degree dependent upon this (*cf.* below). Thus *p*7 (like *p*6) gives dwarf hybrids, while *p*30 gives vital ones, and this character is Mendelian; more on this point later. The hybrids between *L. intermedium* and tetraploid *L. purpureum* during 1944 were obtained after crossing *i*22 with *p*7, while those during 1945 — including the aberrant plant — were *i*47×*p*30. It is probably not necessary to attach any importance to the difference in the mother lines, firstly because *L. intermedium* has proved to be a very homogeneous species (*cf.* BERNSTRÖM, 1953 b), and secondly because the plant development in hybrids between *L. hybridum* and tetraploid *L. purpureum* has proved to depend entirely upon the *L. purpureum* line used. The plants of *p*30 that were used for the fertilization of *i*47 were in part in all probability offspring of spontaneous hybrids with *p*6 or *p*7 (*cf.* BERNSTRÖM, 1954). The difference in development as between the hybrid plants of *L. intermedium*×tetraploid *L. purpureum* during 1945 may thus very conceivably be due to a different effect of *p*7 (or *p*6) and *p*30. Allohexaploids between tetraploid *L. purpureum* and diploid *L. amplexicaule* may bear out this suspicion (*cf.* p. 63). Subsequent crossing experiments intended to throw light upon this problem have failed.

B. L. intermedium×tetraploid L. amplexicaule

a. *Cytology*

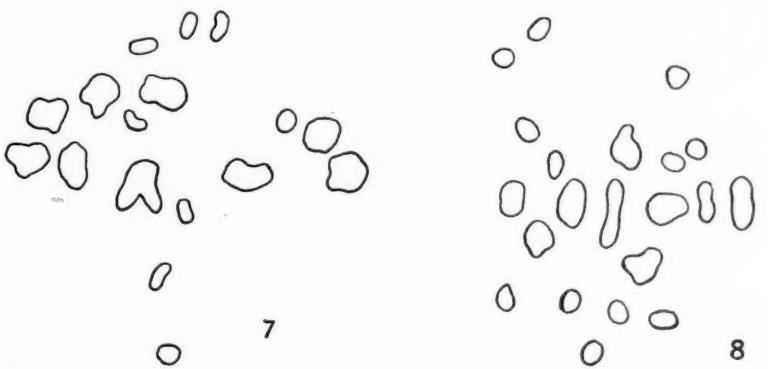
It has been possible to analyse cells in I M from four slides of *a*16×*i*22, and reciprocally. As for *L. intermedium*×tetraploid *L. purpureum*, it has not been possible to draw any definite distinction between the different kinds of configuration. The results of the studies are summarized in Table 2.

Here, as in the case of *L. intermedium*×tetraploid *L. purpureum*, the

TABLE 2. *Chromosome pairing at I M in hybrids between L. intermedium and tetraploid L. amplexicaule.*

Per cell / Slide No.										Number of cells
	I's	8	9	10	11	12	13	9	10	
	Conf's	9	9	9	9	9	9	10	10	
1		6	21	9	7	1	—	2	—	46
2		4	8	5	2	1	1	—	2	23
3		1	17	5	5	—	1	—	1	30
4		2	6	2	1	2	—	2	—	15
	Total	13	52	21	15	4	2	4	3	114

trivalents proved to be the quite obviously dominating type of configuration. Further, 9 proved, also in these hybrids, to be the commonest number of both univalents and configurations. The numerical relations were, as a matter of fact, very similar to those in *L. intermedium*× tetraploid *L. purpureum* (*cf.* Table 1). As in the latter, single quadrivalents occurred also in *L. intermedium*×tetraploid *L. amplexicaule*, as could be observed directly and inferred from the occurrence of cells with 9 configurations+8 univalents; nor were any configurations seen here which might justifiably have been interpreted as pentavalents. Fig. 7 shows a cell with 8 I+9 configurations, of which one (thickly V-shaped) is probably the quadrivalent. Fig. 8 represents a cell from



Figs. 7—8. I M in *L. intermedium*×tetraploid *L. amplexicaule*. — Fig. 7, 9 configurations+8 I, the V-shaped configuration probably constituting a IV; Fig. 8, probably 6 III+3 II+12 I. See the text. The chromosomes separately drawn in both figures. — ×3000.

another slide with probably 6 III+3 II+12 I; it has been drawn to illustrate the difficulty one sometimes finds in distinguishing also bivalents and trivalents. The cell in Fig. 7 is seen obliquely from the side, and in both figures the chromosomes have been separately drawn, with their respective levels retained.

The predominant occurrence of trivalents among the configurations and the circumstance that these are very often nine together with nine or somewhat more univalents lead one to the conclusion that one of the genomes from *L. intermedium* must be homologous with those from *L. amplexicaule*. Conditions are quite analogous with those found in *L. intermedium* × tetraploid *L. purpureum*; and as with the latter the homology between the genomes, also in the present hybrids, must be presumed to be very good, as far more than half of the cells have only 8 to 9 univalents. We shall revert to these questions later.

Corresponding to the case with *L. intermedium* × tetraploid *L. purpureum*, the occurrence in *L. intermedium* × tetraploid *L. amplexicaule* of quadrivalents, as also the occurrence of cells with 10 configurations consisting of trivalents and bivalents, indicates either partial homology between the univalent-forming genome and the other three genomes, or structural divergences between the line of *L. amplexicaule* used and the genome from *L. intermedium* homologous with the latter's genomes, or both. Here, too, it holds good that the cells with 9 configurations + 8 I must be explained by the occurrence of partial homology between the two kinds of genomes. Even if one must make a certain reservation for the number of such cells, assuming that the regular 9th univalent has occasionally been lost in one way or another, they are nevertheless far too numerous and distinct for it to be possible to mistake them.

b. *Fertility and morphology*

Hybrids of five different line combinations have been produced, of which two have comprised reciprocal hybrids. There were no differences between reciprocal hybrids, any more than in any other cross combination in *Lamium*. Between hybrids from different line combinations there were only very slight differences in appearance; these were due to differences between the *L. amplexicaule* lines, as there are scarcely any differences between the lines of *L. intermedium* (*cf.* BERNSTRÖM, 1953 b).

The hybrids were predominantly reminiscent of *L. intermedium* in appearance, and very little of *L. amplexicaule*; *cf.* Fig. 9. Several minor features in, *inter alia*, the final lengths of the internodes, the shape of

Fig. 9. To the left tetraploid *L. amplexicaule* (*a*16), to the right *L. intermedium* (*i*22), in the centre their F_1 hybrid.

the leaves and the calyces do, however, distinguish them from *L. intermedium*. As regards the flowers, tetraploid *L. amplexicaule* has both chasmogamic and cleistogamic ones, whereas the flowers in *L. intermedium* are all chasmogamic. The hybrids had both chasmogamic and cleistogamic flowers, and in addition to this a continuous series of transitional forms between these two kinds of flowers. The extreme types were numerically predominant. The chasmogamic flowers in the hybrids were much more like those of *L. intermedium* than those of *L. amplexicaule*.

In view of the predominant occurrence of trivalents and univalents in I M the fertility of the hybrids may be expected to be low. The pollen fertility varied between 6 and 47 %, and was on an average about 20 %. It was often difficult to decide whether filled grains should be reckoned as fertile, since they varied greatly in respect of both stainability and size. As long as such pollen analyses are not combined with at least the germination of the pollen *in vitro*, it stands to reason that estimations of the 'fertility' must be very uncertain. — The seed-setting varied between 0—7 seeds per plant (the average for a generation was 3,6 seeds per plant). The seed-setting would probably have been better,

if the self-fertilization had not failed in a large number of the flowers. An investigation showed that this was the case with about 70 % of the open flowers, and the cause was found to be poor co-ordination in different ways between the stamens and the stigma.

C. L. hybridum × tetraploid L. purpureum

1. *Tetraploid hybrids*

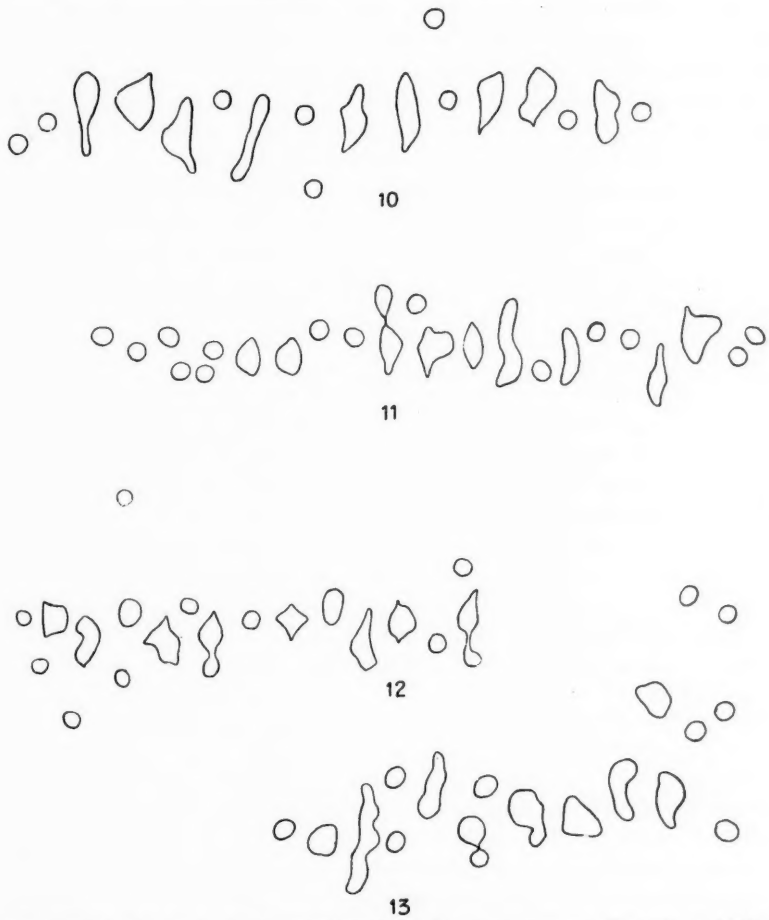
a. Cytology

As will be described in greater detail below, the F_1 hybrids are characterized either by a completely normal plant development, or by pronounced dwarfishness. Cytological investigations have been possible only with plants of the first-mentioned kind. Despite the fact that 3 different fixing agents (»Flemming-Benda», »strong Flemming» and »La Cour 2 BE»; *cf.* DARLINGTON and LA COUR, 1942) and a varied fixing technique were tried, it was not possible to obtain slides clear enough to permit with any degree of certainty of a thoroughgoing distinction of the different kinds of chromosome configuration. Thus here, too, it was unfortunately necessary to use the mode of classification in I's+configurations applied with other species hybrids.

In the same way as in the two above-discussed tetraploid species hybrids, univalents and trivalents have in the hybrids between *L. hybridum* and tetraploid *L. purpureum*, to judge from the appearance of the configurations, proved to be numerically predominant as compared with bivalents and quadrivalents. In view of the fact that quadrivalents evidently occurred more as an exception than as the rule, it emerges also from the number of univalents+configurations per cell, and from the frequencies of the different kinds of chromosome conjugations, that

TABLE 3. *Chromosome pairing at I M in hybrids between L. hybridum and tetraploid L. purpureum.*

Slide No.	Cross combinations	I's / Conf's	9 / 9	10 / 9	11 / 9	12 / 9	13 / 9	14 / 9	15 / 9	8 / 10	9 / 10	10 / 10	11 / 10	Number of cells
1	*h*21×*p*42		6	5	8	11	10	2			3	1	2	48
2	*h*21×*p*30		10	16	12	10	2	3				1	1	55
3	»		14	19	20	19	6					2	1	81
4	»		4	11	5	7	3		1	1		1	2	35
5	»		8	12	15	6	2				2	4	1	50
		Total	42	63	60	53	23	5	1	1	5	9	7	269



Figs. 10—13. I M in *L. hybridum*×tetraploid *L. purpureum*. — Fig. 10, probably 9 III+9 I; Fig. 11, probably 4 III+5 II+14 I; Fig. 12, probably 7 III+3 II+9 I; Fig. 13, probably 1 IV+7 III+1 II+9 I. The chromosomes are separately drawn in all figures. See the text. — ×2500.

trivalents must have been the commonest configuration (*cf.* Table 3). From this it appears that the hybrids between *L. hybridum* and tetraploid *L. purpureum* had on the whole, in the same way as the above-discussed hybrids, three homologous and one odd genome, and that *L. hybridum* must consequently be an allotetraploid.

The occurrence of 10 configurations in about 8 % of the cells must imply either partial homologies between the chromosomes of the threefold (more or less) and the odd genome, or structural divergencies between the chromosomes in the threefold genome, or both alternatives; they may be closely connected. The material does not permit of a more definite opinion on the point. The occurrence of 10 configurations per cell, however, points to chromosome-structural conditions enabling the appearance also of quadrivalents. If there is partial homology between the threefold and the odd genome, we may assume that the chromosomes from *L. purpureum* in the former are those chiefly affected, for no trivalents or quadrivalents occurred in pure *L. hybridum*, and very few univalents (*cf.* BERNSTRÖM, 1953 b). Such an indication of structural difference between *L. purpureum* and the one genome of *L. hybridum* is, however, vague. No species in the genus that is taxonomically close to *L. purpureum* is known, and as the homology between the one genome of *L. hybridum* and the genomes of *L. purpureum* is on the whole very good, one may from these two circumstances assume for the time being that *L. purpureum* is the one parent species of *L. hybridum*. Cytogenetic investigations do not contradict such an assumption (*cf.* below).

Some observations have been made which indicate that the chiasma frequency in hybrids between *L. hybridum* and tetraploid *L. purpureum* may be considerably affected by the temperature; the investigations on this point need, however, to be supplemented, and the observations to date in these respects do not alter anything essential in the conclusions drawn above.

Figs. 10—13 show the chromosomes at I M in four PMC's of the hybrids; in all of these the chromosomes have been separately drawn with their respective levels retained. Figs. 10—11 are from slide No. 1 (fixed in Flemming's fixing agent). The former probably shows 9 III+9 I, while the latter probably has 4 III+5 II+14 I. Fig. 12 is from slide No. 5 (fixing agent: Flemming-Benda) and has probably 7 III+ +3 II+9 I, and Fig. 13 is from slide No. 3 (Flemming-Benda) and probably shows 1 IV+7 III +1 II+9 I.

b. Morphology, and genetics of hybrid dwarfishness

The hybrids between *L. hybridum* and tetraploid *L. purpureum* have — with one exception; *cf.* below — proved to develop either into fully

grown and normally developed plants, or into pronounced dwarf forms. The difference between the two kinds of hybrids is very great. The following survey shows with what lines of *L. hybridum* (acting as mother) the different lines of tetraploid *L. purpureum* have been hybridized. The lines of *L. hybridum* used have been presented in a previous work (BERNSTRÖM, 1953 b); the same applies to the lines of *L. purpureum* (BERNSTRÖM, *loc. cit.*, 1954).

Lines of tetraploid *L. purpureum*	Diploid line of origin	Origin	Lines of *L. hybridum* with which hybrids have been produced	Type of hybrids
*p*6	*p*1	Gothenburg, Sweden	*h*19	dwarfs
*p*7	*p*2	ditto	*h*18, *h*19, *h*21, *h*36, *h*37	»
*p*30	*p*25	Lund, Sweden	*h*18, *h*21, *h*36	normal
*p*42	*p*26	Waadt, Switzerland	*h*21, *h*36	»
*p*43	*p*29	Malmö, Sweden	*h*18, *h*37	dwarfs
*p*59	*p*51	Lund, Sweden	*h*49	normal
*p*60	*p*53	ditto	*h*49	»
*p*61	*p*54	ditto	*h*21, *h*37, *h*49, *h*89	dwarfs
*p*62	*p*66	ditto	*h*37, *h*49	»
*p*90	*p*71	Sverdlovsk, Russia	*h*37	normal
*p*101	*p*96	Koralpe, Austria	*h*37	»
*p*102	*p*97	Antwerp, Belgium	*h*37	dwarfs
*p*103	*p*98	Rouen, France	*h*37	(not fully) normal
*p*104	*p*99	Graz, Austria	*h*37	normal

It emerges from the survey that the type of the hybrids is obviously determined by the *L. purpureum* line used and is independent of the line of *L. hybridum*. The same lines of *L. hybridum* which with one line of *L. purpureum* give normal hybrids give dwarf plants with another. Different lines of *L. hybridum* can modify the type of growth of the hybrids only to a certain extent, but they cannot decide the development into dwarfs *contra* fully vital plants (*cf.* below).

For all the line combinations above, with one exception, only *L. hybridum* served as mother. Owing to the difficulty of producing hybrids between *L. hybridum* and tetraploid *L. purpureum* with the latter as mother, such hybrids were studied only in the combination *p*62×*h*37 (*cf.* BERNSTRÖM, 1953 a). They proved to be typical dwarf plants of exactly the same type as after the reciprocal crossing *h*37×*p*62; and, at least in this case, the dwarfish growth is thus not an effect produced by the joint action of the plasmon of *L. hybridum* and the genotype of the *L. purpureum* line in question.

It has not been possible to trace any common external characters of those lines of tetraploid *L. purpureum* which through the kind of hybrids with *L. hybridum* constitute the respective groups. Nor is any clear differentiation between them in respect of the origin of the lines traceable. It has been pointed out earlier that the property of segregating sterile dwarfs in diploid interlinear F_2 progeny within *L. purpureum* cannot be due to the same genes that produce dwarf development in hybrids with *L. hybridum* (BERNSTRÖM, 1953 b). Nor could any other kind of genetic differentiation be observed between the two hereditary types of lines in an investigation of the plant development and the fertility of a large number of diploid F_1 hybrids. Thus the property of causing dwarfishness in hybrids with *L. hybridum* seems to be about as common as that of permitting the hybrids a fully normal development; and as far as the investigations have been taken, this differentiation of *L. purpureum* has not been found to be connected with any other kind of genetic or geographical division of the species.

The normally developed type of hybrids between *L. hybridum* and tetraploid *L. purpureum* reminds one, as a rule, in appearance somewhat more of the former than of the latter species; *cf.* Fig. 14. In the different characters the hybrids are more or less intermediary, *e. g.*, in the shape and indentation of the leaves, the form of the flowers and calyces, and the size of the flowers. These, during the warmer part of the year, are chasmogamic, and in the autumn they do not change to closed, pseudocleistogamic flowering until this takes place to the same extent also in the parent lines of *L. hybridum* (*cf.* BERNSTRÖM, 1953 b, p. 397). Self-fertilization does not function as reliably as in the parent species, but it is clearly somewhat better than it otherwise generally is in species hybrids within the genus.

The hybrids are characterized by a thriving vegetative development. Where it has been possible to compare hybrids and parents with reference to the degree of development, the hybrids have very definitely proved to be larger than any of the pure species. They have also shown a more rapid development, and they begin flowering earlier than the parents.

It has been indicated above that $h37 \times p103$ did not agree with the normally developed type of hybrids. The divergence lay in a more richly branching but not so tall growth, which otherwise characterized the vital hybrids, and in a slight reduction in the size of leaves, and a change in the shape of leaves. Although not strongly pronounced, these characters were the same as those typical for the medium-sized se-

gregates of *L. hybridum*×tetraploid *L. purpureum* (*cf.* below); between these and typical dwarfish hybrids there were a number of transitional forms. The hybrids between *h*37 and *p*103 thus proved to diverge from well-developed hybrids of other line combinations in the possession of features pointing to the dwarf type.

The dwarfish hybrids are always very different from the normal ones, not only in size but also in the whole type of growth, which diverges markedly from that found in even very small plants of the pure species. The size of the hybrids and the degree of their branching depend both upon the genotypes of the parent lines and upon the season; semi-shade and a moist atmosphere favour the development of the plants, which thus tends to be better in the autumn than in the summer. Independently of the seasonal variation, *etc.*, however, the difference in size and type of growth as between normally developed hybrids and dwarfs is always very considerable. The embryos which give rise to dwarf hybrids have proved to be of the same size and just as well developed as the embryos resulting in normal hybrids. With the development of the second pair of leaves, but hardly before, it is possible to distinguish the dwarf plants from the normally growing hybrids.

Characteristic features in the dwarf plants are irregular branching, very often marked by the lack of a real or substitute, dominating stem, and unevennesses in the size, shape and indentation of the leaves, both on and between the branches. Thus the pyramidal form with respect to the size of leaf in the inflorescences of stem and branches that is typical for the genus and the family is very often lacking, the leaves in the second or third whorl, counting from below, generally being the biggest. Leaves and branches on especially the very small dwarfs are rather stiff and brittle. This, together with the irregularities in the growth, indicates that the plants are suffering from physiological disturbances.

The flowers are much smaller than those in the normally developed hybrids; in the summer they are as a rule chasmogamic, but in the autumn for the most part closed and very small. The pollen production, even in the most well-developed flowers, is not so good as in the hybrids of normal type, and it becomes poorer with diminishing size of flower.

Fig. 15 shows a hybrid plant of the combination *h*37×*p*7 between the parent lines. Fig. 16 reproduces a plant of *h*19×*p*7, cultivated in the summer, and Fig. 17 a plant of the same combination, cultivated in the early autumn. The difference between the plants, as this appears from the picture, indicates the effect of the season. The hybrid plants re-

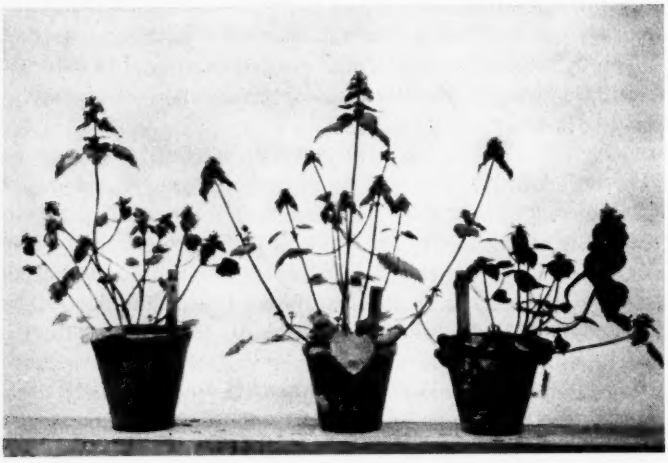


Fig. 14. To the left *L. hybridum* (*h*36), to the right tetraploid *L. purpureum* (*p*30), in the centre their F_1 hybrid.

Fig. 15. To the left *L. hybridum* (h*37*), to the right tetraploid *L. purpureum* (*p*7), in the centre their dwarfish F_1 hybrid.

Fig. 16. Dwarfish F_1 hybrid between *L. hybridum* and tetraploid *L. purpureum* (*h*19× *p*7), flowering in July. The label is graduated in cm.

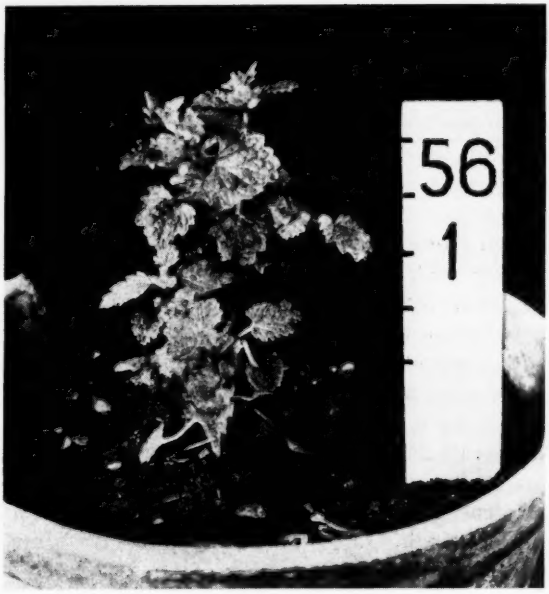


Fig. 17. Dwarfish F_1 hybrid genetically identical with that in Fig. 16, but flowering in September; *cf.* the text. The label is graduated in cm.

TABLE 4. *Kind of F_1 hybrids between L. hybridum and interlinear F_1 hybrids of tetraploid L. purpureum.* (*Cf.* the text.)

Cross combination, and year	Kinds of hybrids, and their number				
	Normal growth	Slightly dwarfish	Intermediate type	Predominantly dwarfish	Dwarfs
1948					
*h*21 × (*p*42 × *p*43)	(43)	(5)		(6)	0
*h*37 × (*p*42 × *p*43)	(11)		(26)	0	9
1950					
*h*21 × (*p*7 × *p*30)	4	0	0	0	1
*h*37 × (*p*7 × *p*30)	5	3	0	19	2
*h*21 × (*p*42 × *p*7)	4	0	1	0	0
*h*37 × (*p*42 × *p*7)	(16)		(13)	0	7
*h*21 × (*p*43 × *p*30)	1	0	0	0	0
*h*37 × (*p*43 × *p*30)	3	0	14	0	8
*h*21 × (*p*42 × *p*43)	(18)	0	0	5	0
*h*37 × (*p*42 × *p*43)	(11)		(33)	0	4
*h*21 × (*p*59 × *p*62)	2	0	0	0	0
*h*37 × (*p*59 × *p*62)	1	2	2	(5)	(4)
*h*21 × (*p*61 × *p*60)	6	0	(2)	0	0
*h*37 × (*p*61 × *p*60)	2	1	0	(23)	(4)

produced in Figs. 15—17 typify the most pronounced dwarfs. Dwarfish growth of a somewhat more thriving type characterized hybrids with other lines, *e. g.*, those with *p*43.

In order to get some idea of the genetic cause of dwarfish and normal growth, respectively, F_1 hybrids between lines of tetraploid *L. purpureum* belonging to each category were crossed with *L. hybridum*. Two lines of the latter were used, with rather different appearance and geographic origin (*cf.* BERNSTRÖM, 1953 b), in order to reveal the effect upon the development of the progeny and therewith upon the possible segregation ratios that the particular genotypes of the parent lines might be conceived to entail. The results of the crossing experiments are summarized in Table 4.

In several of the F_1 generations it was possible to group the plants in five apparently distinct classes, distinguished by the different sizes of the plants and the different degree of characters typifying the dwarf plants which characterized the intermediary plant types. In other F_1 generations, chiefly those with a greater number of plants, there oc-

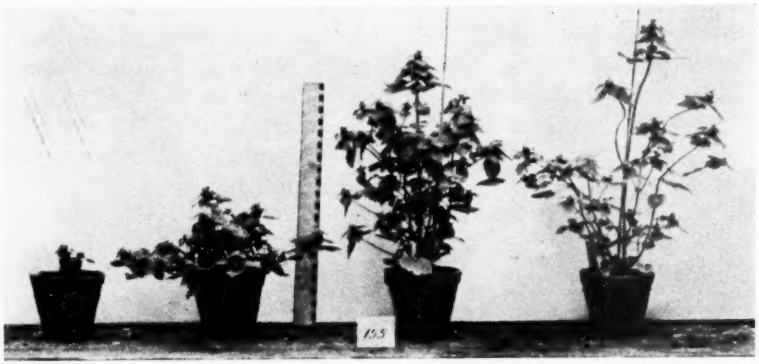
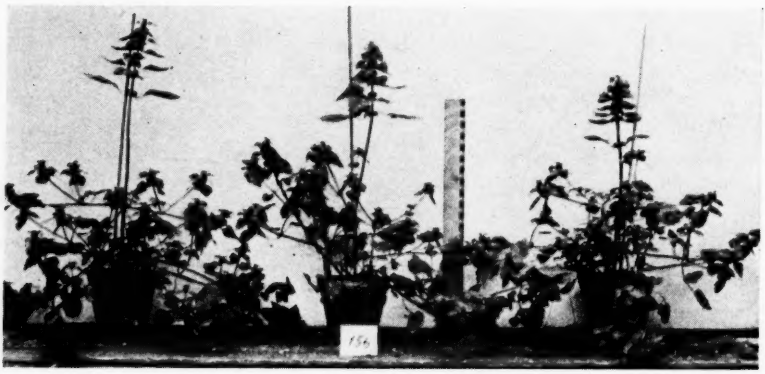


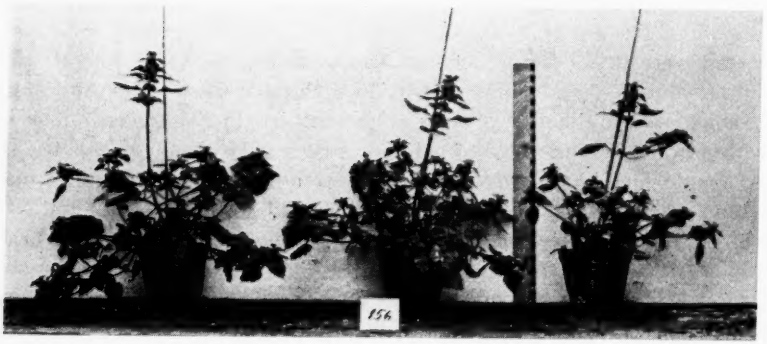
Fig. 18. F_1 sister plants from *L. hybridum*×interlinear F_1 hybrid of tetraploid *L. purpureum* (see the text). The ruler was 40 cm long.

curred transitional forms between two or more classes, so that it was not possible to delimit them clearly. In such cases the number of plants grouped around the nearest class in question has in Table 4 been placed within brackets, and a line of points has been drawn between the classes between which there was no clear difference, or drawn to point out within or between which classes the plants referred to might fall. — Fig. 18 shows a plant of *h*37 to the right together with three hybrid plants from the combination *h*37×[*p*43×*p*30], 1 normally developed, 1 intermediary and 1 dwarf plant, each representing its distinct group of plants. Fig. 19 shows a series of hybrid plants from the combination *h*37×[*p*42×*p*43], representing the successive transition from plants of normal type (the two to the left) to plants of a type approaching what has been called »predominantly dwarfish». On the extreme right is a plant of pure dwarf type.

As appears from Table 4, the two F_1 generations studied during 1948 differed in two respects. In the first place the hybrids with *h*37 included 9 plants of the pure dwarf type, while none such occurred in the progeny after *h*21; and in the second place the hybrids with the latter included a proportionately far lesser number of plants with more or less prominent dwarfish character than those after *h*37. The difference between the progeny is statistically significant ($P < 0{,}001$), both when all the non-dwarfs are contrasted with the dwarfs by computing the χ^2, and when the classes are taken separately, in so far as one may speak of classes at all.



a



b

c

Fig. 19 a—c. F_1 sister plants from *L. hybridum*×interlinear F_1 hybrid of tetraploid *L. purpureum* (see the text). The ruler was 40 cm long.

Owing to this result of the experiment it was repeated, 6 different interlinear F_1 combinations of tetraploid *L. purpureum* of the type »dwarf hybrids×normally developed hybrids with *L. hybridum*» being crossed with the earlier used lines of *L. hybridum*. The number of hybrid plants obtained was unfortunately much smaller than intended, which considerably reduces the value of the experiment. From Table 4, however, it emerges that for the combinations with $p42 \times p43$ the result was about the same as in 1948, so that on this occasion too the combination with *h*37 is the only one showing pure dwarfs, at the same time as, proportionately, it has the largest number of plants in the intermediate classes. This difference between the hybrids from the two *L. hybridum* lines seems, moreover, to recur also in the combinations with the remaining *L. purpureum* hybrids, but is on account of the small number of plants in the combinations with *h*21 clearly apparent only in the combinations with $p61 \times p60$.

On account of the differences in the segregations between combinations with *h*21 and *h*37, respectively, it is *in et per se* difficult to analyse the genic background to the segregations found. If more *L. hybridum* lines than the two selected at random had been used, it is probable that still more widely diverging figures would have been obtained in the progeny. Nor do the small numbers of individuals, which were far fewer than intended, permit of satisfactory analyses of the segregations here in question, since tetraploid segregations require a far greater number of individuals for their analyses than do diploid ones (*cf.* LITTLE, 1945). The occurrence of different types of plant development among the progeny from the crossings shows, however, that the dwarfish character is genically conditioned. Further, the small number of intermediate plants in relation to the number of normal and dwarf plants, respectively, shows, also in the combinations with *h*37, that the genic difference between the two kinds of *L. purpureum* lines may be of a simple nature. The numerical relation between the sum of normally developed and intermediate plants on the one hand, and dwarf plants on the other in all combinations with *h*37 during 1948 and 1950 is $5{,}0 : 1$, which as a matter of fact lies precisely at the upper limit for the theoretically expected numerical relation for one pair of factors in a tetraploid; the lower limit is $3{,}5 : 1$ (MATHER, from LITTLE, *loc. cit.*). With two pairs of factors a far greater preponderance of normal and intermediate plants would have been expected.

If dwarf development is assumed to be due to a single factor in the genome of *L. purpureum*, as regards this species, the varying degrees of

development in the intermediate plants and therewith also the diverging numerical relations between the progeny from *h*37, which with the division given to the plants in Table 4 are statistically significant ($\chi^2=30{,}76$; $P<0{,}001$), may be assumed to be due to secondary genic differences (»modifiers») between the different lines of *L. purpureum*. The, as a rule, small numbers of individuals in the progeny with apparently distinct intermediate classes may conceal the fact that a larger number of individuals would have entailed a more continuous variation. But also the sensitiveness to environment characteristic for *L. hybridum* may conceivably contribute to the variation in the size of the plants (*cf.* BERNSTRÖM, 1953 b, p. 398). F_1 hybrids between *L. hybridum* and *L. purpureo-bifidum*, which were in many respects reminiscent of the intermediate hybrids under discussion, likewise varied surprisingly much in size (*cf.* p. 38).

The above-described features in the hybrids between *h*37 and *p*103, which pointed to the dwarf type, may imply that there are differences between the lines of *L. purpureum* in the effect of the genotypes upon the type of growth in hybrids with *L. hybridum*.

As the male components were the same in the crossings with *h*37 and *h*21, respectively, different kinds of segregations as a cause of the differences in the results between them are scarcely conceivable otherwise than as a consequence of secondary effects. If dwarf development in the hybrids is assumed to be due to a single gene in the *L. purpureum* genomes, the almost total absence of dwarf plants and the lower relative number of more or less dwarfish plants in the progeny from *h*21 may thus conceivably be due to, *e. g.* certation phenomena, selective fertilization, or — perhaps rather — to a greater proportion of abortions among embryos of dwarf type, *etc.*, in the combinations with *h*21 than in those with *h*37. Available data concerning crossings between these two lines of *L. hybridum* with different, pure lines of tetraploid *L. purpureum* giving rise to dwarfs are too scanty to permit of closer investigation of the latter alternative; this also for the reason that frequently a smaller number of hybrid plants were raised than the available amount of seed allowed of, so that some of the seed from cross-pollinated flowers may have been pure. In an earlier paper (BERNSTRÖM, 1953 a) it has been shown that in two combinations giving rise to dwarf plants the compatibility may be very dependent upon the parent lines, although in this case it was a matter of a difference between lines of tetraploid *L. purpureum*, and these served as mothers.

An explanation like the above appears, though unproven, more likely

than the assumption that the genotypes of the respective lines of *L. hybridum* have a considerably different effect on the plant development and the degree of dwarfishness after fertilization with genotypically similar gametes of *L. purpureum*. For the difference in the size of dwarf plants after *h*21 and *h*37, crossed with various lines of *L. purpureum*, has always been slight, whereas the tendency in the differences between the progeny from *h*21 and *h*37, respectively, has in the crossing experiments described been unequivocal as far as it has been possible to draw comparisons by pairs, and also as far as is shown by a comparison of all the progeny deriving from the respective *L. hybridum* lines. — We shall revert below to the question of a genetic explanation of the dwarfishness.

c. Fertility

In the normally developed type of hybrid the pollen seems in part to be rather good, although it is very uneven as to size of grain, as was the case in the species hybrids earlier discussed. However, the quality of the pollen varies somewhat as between different years, as is shown by the following survey of the average percentage of good grains in the hybrid combinations investigated; the number of plants examined is given in brackets:

	1944—2	1945—1	1945—2	1946
*h*21×*p*30			44,1 (10)	
*h*36×*p*30	37,8 (3)	34,6 (5)	38,6 (17)	
*h*21×*p*42			39,2 (10)	25,3 (8)
*h*36×*p*42			38,1 (11)	18,5 (13)
*h*38×F_2 (*p*7×*p*30)				25,5 (23)

Among the dwarf hybrids the percentage of good pollen is intimately connected with the development of the flowers (*cf.* p. 17). The larger, more open and in this case also better coloured these are, the better is the pollen fertility. The development of the flowers is in its turn dependent upon the vegetative development of the plants, and upon a moderate supply of light and probably also warmth. In favourable circumstances the pollen fertility, which in all cases was investigated only in samples from open flowers with burst anthers, is as high as it generally is in normally developed hybrids, *i. e.*, 35—40 % of the grains are good. In more unfavourable circumstances the corresponding figures are under 5 %, whatever the material. We shall revert later to this matter (pp. 90—91).

The seed-setting in the normally developed hybrids is very poor. In 1945—2 a careful investigation was made of the seed-setting in the four hybrid combinations in the survey below. The plants were examined several times in the course of the flowering, and scars from fallen seed were also noted. The average number of seeds per plant was found to be:

$h21 \times p30$	$9{,}50 \pm 1{,}035$	10 plants
$h36 \times p30$	$6{,}18 \pm 0{,}787$	17 »
$h21 \times p42$	$4{,}30 \pm 0{,}817$	10 »
$h36 \times p42$	$4{,}73 \pm 1{,}145$	11 »

The causes of the in part statistically significant differences in the extent of the seed-setting are probably scarcely to seek in meiotic differences. I M, at all events, as Table 3 shows, agrees very closely in the most and the least seed fertile combinations — $h21 \times p30$ and $h21 \times p42$, respectively —, and the fixations of slides Nos. 1—5 were performed in close succession. Further, as has already been pointed out, intraspecific F_1 hybrids between both the *L. hybridum* and *L. purpureum* lines in question are fully fertile. Nor can the differences in the seed-setting of the hybrids be due to the greater or lesser number of flowers, for if the hybrid combinations had differed from each other in this respect — which was not in any degree worth mentioning the case —, then $h36 \times p42$ should rather have had the best seed-setting, for the plants from this combination were the largest. The differences in the seed fertility are instead due rather to genic causes, for also the F_2 after the two best seed-fertile F_1 combinations, which constituted crossings with $p30$, proved to have better seed-setting than the two others (*cf.* Table 17). The question will be dealt with in greater detail in connection with the discussion of the F_2.

The extent of the seed-setting in the dwarf hybrids is in a high degree dependent upon the way in which the flowers have developed. As these are, as a rule, small and closed or almost closed, with very small and generally also closed anthers, the hybrids are in consequence, also as a rule, completely seed-sterile. That the cause of the seed sterility is to seek here is shown by the fact that where thanks to suitable weather conditions and suitable placing the plants have been able to develop well in the summer, the flowers and their anthers are bigger, and the latter open normally. The seed-setting in such plants is relatively quite equal to that in the normally developed hybrids; thus, the average numbers of seeds per plant was, for seven unusually well-developed plants from $h21 \times p7$, 6,0.

2. *Octoploid hybrids*

Before the obvious cause of sterility constituted by the preponderant occurrence of trivalents and univalents at I M among hybrids between *L. hybridum* and tetraploid *L. purpureum* was known, the number of chromosomes in F_1 hybrids between *h*36 and *p*30 was doubled through colchicine treatment, in order to investigate the effect of the doubled number of chromosomes upon the seed-setting. A very marked improvement in the latter was the result. Seven on the whole octoploid plants in C_1 had on an average a seed-setting of 13,3 %, while for the corresponding tetraploid hybrids during the same generation it was 6,2 seeds per plant, which corresponds to only about 0,2 %, as the number of flowers per plant was approximately 750 (calculated for 6 individuals). Thus the seed fertility of the octoploids was more than 60 times better than that of the tetraploids, and this despite the fact that the self-pollinating capacity of the octoploids was obviously less than that for the tetraploids, as a consequence of the large number of chromosomes.

This improvement in the seed-setting must be due to the possibilities of more regular chromosome pairing afforded, resulting in a generally improved vitality of the gametes in consequence of a better chromosomal balance. Thus the sterility of the tetraploid hybrids is probably at least for the most part chromosomal and not genic (the terms from DOBZHANSKY, 1951). The seed-setting in what was in that connection the best octoploid line of *L. hybridum* proved, moreover, to be of about the same order of magnitude as for the octoploid hybrids; 22 plants in C_1 had an average seed-setting of 19,3 % (BERNSTRÖM, unpubl.).

During two subsequent generations the octoploid hybrids suffered a very pronounced impairment in the seed-setting; thus, this was on an average in C_2 3,4 % for 11 plants and in C_3 1,0 %, likewise for 11 plants. The pollen fertility, on the other hand, remained constant. For C_1 it was 59,6 %, for C_2 56,9 % and for C_3 61,6 %. The two individuals with the highest seed fertility in each generation were made to serve as mother plants for the subsequent generation.

No investigations of the number of chromosomes were carried out in C_2 and C_3. However, the octoploids of two different lines of *L. hybridum* showed also a gradually increased reduction in the seed fertility reminding one of that in the octoploid hybrids, but likewise a retained pollen fertility, which varied between 75 and 90 %. From having been, already in C_1, rather variable but preponderantly hypo-octoploid, the number of chromosomes rapidly became increasingly reduced, so that in C_2 and C_3 ± heptaploid individuals were numerous, or predominant.

A reduction in the number of chromosomes increasing with the generations may be expected to occur more readily in the octoploid hybrids between *L. hybridum* and *L. purpureum* than in octoploid *L. hybridum*, since the former, as we know, have 6 at least on the whole homologous genomes, while the latter have 2 groups each with 4 homologous genomes. There are thus strong reasons for the assumption that the diminishing fertility in the octoploid hybrids in C_2 and C_3 was due to an increased chromosomal unbalance through the increasing elimination of especially those chromosomes which in the tetraploid hybrids form trivalents.

Morphologically and physiologically the octoploid hybrids manifest the high chromosome number very clearly in a coarsening of the different organs, fewer and shorter branches, slower development, *etc.*, all familiar features in different kinds of plant material with artificially increased chromosome number. The morphological variation between the plants was very marked. The development of the material was, however, about the same in all generations, which probably constitutes still another illustration of the fact that plant vigour does not so soon suffer from aneuploidy as the seed-setting, as was found to be the case in tetraploid *L. purpureum* and *L. amplexicaule* (BERNSTRÖM, 1954).

II. Diploid and triploid F_1 hybrids, their corresponding allopolyploids, and hybrids with them

A. L. purpureum × L. bifidum subsp. balcanicum

1. *The diploid hybrids*

After the following crossing experiments twenty-four hybrid plants were obtained between the diploid line *p*53 of *L. purpureum* and *L. bifidum* subsp. *balcanicum* (*cf.* p. 3):

*p*29 × *L. bifidum* subsp. *balcanicum* 21 flowers cross-pollinated; 0 seeds obtained,

*p*53 × *L. bifidum* subsp. *balcanicum* 31 flowers cross-pollinated; 30 seeds obtained,

L. bifidum subsp. *balcanicum* × *p*29 19 flowers cross-pollinated; 0 seeds obtained,

L. bifidum subsp. *balcanicum* × *p*53 16 flowers cross-pollinated; 0 seeds obtained.

Cross-pollination of *L. bifidum* subsp. *balcanicum* never resulted in ovules that were even swollen; and this, together with the negative

result of the cross-fertilization of *p*29, indicates both that the compatibility between the two species may be one-sided, and that the cross-ability in the fertile crossing direction may be regulated by characters or genes peculiar to the lines in question. The possible import of this will be discussed below.

The majority of the hybrid plants were treated with colchicine in order to double the chromosome number; the remainder developed into tall, richly branching plants characterized by marked hybrid vigour. They developed as quickly as *p*53 and commenced to flower at about the same time as the latter; the flowering was rich. *L. bifidum* subsp. *balcanicum*, on the other hand, which was sown in a greenhouse at the same time as the others on the 15/6, grew very slowly, on account, it may primarily be assumed, of winter annuality; and on the 1/9, when the flowering in the others was coming to an end, it was still standing in the form of small, non-flowering plants with many leaves. Thus the summer annuality of *p*53 showed in the hybrids complete dominance over the refusal of *L. bifidum* subsp. *balcanicum* to flower the same summer as it was sown. This is of interest in connection with the question as to the origin of *L. hybridum*; as this species has always proved to be summer annual, we may assume that at least one of its parents was also summer annual, though not necessarily the other.

As regards especially the shape of the leaves, but also of the calyces, the hybrids reminded one in several respects of *L. hybridum*. The flowers were small, scarcely longer than the calyx, and chasmogamic only during the first part of the flowering period. The anthers were small, poor in pollen and sometimes closed. Not a single seed was set and not even swellings of ovules were observed, which might have indicated that fertilizations had taken place. Out of about 2000 pollen grains from 5 plants only 4 were good.

In spite of extensive fixation of buds for study of the meiosis, no usable slides were obtained; the poor development of the anthers was probably partly responsible for this. Thus, the degree of pairing in I M between the genomes of the parent species is unfortunately not known.

2. *Allotetraploid L. purpureo-bifidum*

a. Cytology

The treatment of the diploid hybrids with colchicine resulted in several fertile branches. The progeny from 4 of these were bred, and

TABLE 5. *Frequencies of configurations and univalents in I M of L. purpureo-bifidum.*

Per cell	Plant No. 1	2	3	4	n
1 III + 16 II + 1 I		2		1	3
18 II	10	41	21	56	128
17 II + 2 I		1	2	3	6
16 II + 4 I		1			1

consisted exclusively of tetraploid plants. For the sake of clarity, the thus formed allotetraploid, which has ever since remained constant (*cf.* below), will in the sequel be referred to as *L. purpureo-bifidum.*

It has been possible to study the reduction division in four C_1 plants from three of the families mentioned. Data concerning the chromosome pairing in I M are given in Table 5.

In two microsporangia in plant No. 4 there occurred one cell each with ± 36 bivalents. Such a case of syndiploidy has been observed in *L. intermedium* (BERNSTRÖM, 1953 b). Further, in plant No. 2 one cell had 1 bivalent and 34 univalents; this was situated at the inner wall of a microsporangium in which all the other cells had 18 bivalents. The asyndesis may have been caused by the position of the cell, and is probably to be regarded as an abnormality without particular significance.

Thus, in the great majority of cells are regularly formed 18 bivalents. A comparison with the corresponding data for *L. hybridum* and *L. intermedium* (*cf.* BERNSTRÖM, 1953 b, p. 402 and Table 3) shows that in the former the only irregularities consisted in the fact that 4 % of the cells had 2 univalents and 17 bivalents, whereas *L. purpureo-bifidum* had 2 to 4 univalents in 5 % of the cells, in addition to which were found one trivalent + one univalent in > 2 % of the cells. The aberrations from the normal bivalent pairing in the new allotetraploid were thus only slightly more in number than in the stable species *L. hybridum.* They were, on the other hand, far fewer than in *L. intermedium*, which — if the column farthest to the right is used — had a multivalent in > 1 % of the cells, and 2 or more univalents in as many as 23 % of them. *L. purpureo-bifidum* thus proved quite able to measure up to its nearest allotetraploid relatives in the regularity of bivalent formation.

In plant No. 3, 75 cells in I A showed no irregularities, while one had a bridge between the groups. No fragment was observed. In II M from the same plant 81 cells showed a normal distribution of the chromo-

somes, the distributions always being 18—18 when observable. In two cells, on the other hand, one chromosome lay outside one of the plates. In II M in another plant 71 cells likewise appeared normal, while in another cell a bridge had possibly been formed between the plates. In II A in two plants 65 cells showed no irregularities, but only in exceptional cases was it possible to check the numbers of daughter chromosomes.

The reduction division in *L. purpureo-bifidum* thus seems to take place with few irregularities. The occasional trivalents must imply the existence of a degree of homology between the two kinds of genomes making possible incidental pairing and chiasma-formation between them. The cause is probably the same for at least the majority of univalent pairs. Since *L. purpureo-bifidum* is rather fertile (*cf.* below), one can from the regular formation of bivalents and with examples from the origin of several well-known allotetraploids conclude that the sterility in the diploid hybrids was chromosomal, and that only a low degree of chromosome pairing took place.

b. Fertility and morphology

Pollen fertility and seed-setting have been investigated in plants in C_1—C_8 and at the same time one line each of *L. intermedium* and *L. hybridum* has been used as a measure of the fertility of the natural allotetraploid species. As emerges from Table 6, the quality of the pollen in *L. purpureo-bifidum* is quite as good as that of the two natural species, while the seed-setting, on the other hand, is 15—30 % below theirs.

The step from the total sterility of the diploids to the rather good seed fertility of the tetraploids is in itself, of course, a wonderful change, and *L. purpureo-bifidum* constitutes a new and good example of the old familiar instances of the way in which a sterile hybrid may become fertile through a doubling of the chromosome number. On account of its recency one may not expect a seed-setting as good as that in the natural allotetraploids, of which probably *L. hybridum*, more than *L. intermedium*, has had the possibility of undergoing the fertility-favouring development that in all likelihood may be offered by mutations, and by incidental interlinear hybridization with subsequent recombinations. Intralinear recombinations through heterogenetic pairing were also to be expected in allotetraploids (STEBBINS, 1950); however, the cytological homogeneity of both species argues against its occurrence here.

As the meiosis in *L. purpureo-bifidum* has proved to be on the whole

TABLE 6. *Pollen fertility and seed-setting in L. purpureo-bifidum, L. intermedium and L. hybridum.*

Species, or equivalent	Measures of fertility	1951			1953			1954		
		C-generation	$\bar{x}$	n	C-generation	$\bar{x}$	n	C-generation	$\bar{x}$	n
L. purpureo-bifidum	pollen fertility	C_1	97,9	38	C_2	99,4	16	C_3	99,8	20
	seed-setting		57,7	39		58,8	16		59,4	21
L. intermedium (*i*39)	pollen fertility	—	98,4	9	—	99,1	8	—	99,6	10
	seed-setting		79,9	9		68,1	8		88,6	10
L. hybridum (*h*37)	pollen fertility	—	97,3	10	—	—	—	—	99,4	10
	seed-setting		82,9	8		71,1	10		86,8	10

regular, the reduction of the seed-setting to about 60 % of the numerically possible must to a predominant extent be caused by other factors than chromosomal disturbances. The formation of gametes, the fertilization and the seed development are among the most delicate of the life-processes in plants, and are normally regulated by gene systems, which through long selection have attained to a fine balance. In a new allotetraploid the balance is disturbed, firstly by the doubling of the chromosome number *per se*, for which the genotypes are not adapted, and through the anatomical and physiological changes entailed in the doubling, and secondly by the fact that two different gene systems are forced to co-operate. There are instances of new allotetraploids becoming markedly sterile in consequence of, *inter alia*, genic disturbances between the two genomes, (*cf.*, *e. g.*, CLAUSEN, 1941; SEARS, 1941).

There are, on the other hand, reasons for the assumption that as against the deleterious effect upon the fertility of a doubled chromosome number, and possibly of unfavourable complementary genes and unbalanced chromosome numbers, we may reckon with the counteracting influence of positive forces of heterotic nature. F_1 hybrids between lines of new autotetraploids are often characterized by a far better seed-setting than that of the parents, through an effect which should be compared with heterosis and which may also characterize the pollen fertility (BERNSTRÖM, 1954). F_1 hybrids between lines of diploid *L. purpureum* are distinguished by, *inter alia*, better seed-setting than that of the parents, probably in consequence of a more reliable self-fertilization as a result of heterosis (BERNSTRÖM, 1950 a). Such fertility-favouring effects of crossing may be expected to occur in interspecific just as well as in interlinear hybrids, though they may be hidden by partial sterility, and may be difficult to measure for technical reasons. Allotetraploids offer good opportunities for studies of this kind of »reproductive heterosis» (term from GUSTAFSSON, 1951), which in these may also probably be of evolutionary significance through the fixation of heterosis characterizing allopolyploids (EAST, 1936; HAGBERG, 1953; *et al.*). This is to say that if the degree of structural inhomology between the genomes of two species is sufficiently great to assure their allotetraploid a degree of heterogenetic pairing which is harmless for its fertility, and there exist no unfavourable complementary gene combinations, then the degree of reproductive heterosis in allotetraploids formed by different lines *etc.* of the species may be of importance for the possibilities of survival of these allotetraploids.

As regards the case in point, *p*60 (*i. e.*, the tetraploid line of *p*53,

which constitutes the *L. purpureum* component in *L. purpureo-bifidum*) was cultivated in the same year as C_1 of *L. purpureo-bifidum*, but unfortunately it was possible to determine only the pollen fertility; this amounted to 97,8 % for 8 individuals, *i. e.*, the same as for the allotetraploid. The only available value of the seed-setting for *p*60 dates from 1949, which was a very favourable year for the seed-setting in all tetraploid lines of *L. purpureum*; the seed-setting in *p*60 was then 30,8 %. Thus, the seed-setting in *L. purpureo-bifidum* was almost twice as high as in the autotetraploid of the one parent, to judge from the available values; and of tetraploid *L. purpureum* only a couple of the best interlinear hybrids showed an equally good seed-setting (BERNSTRÖM, 1954). — The seed-setting in *p*53 was for two different years 93,1 % and 98,8 % (BERNSTRÖM, 1950 a).

As long as the seed-setting and the meiosis in autotetraploid *L. bifidum* subsp. *balcanicum* have not been studied, nothing definite can be said concerning the causes of the good fertility shown by *L. purpureo-bifidum*. It does not seem likely to me, however, that the almost regular bivalent pairing in the allotetraploid is the most essential cause of the latter's considerably better seed fertility as compared with that of *p*60, this because an increased bivalent formation in interlinear F_1 hybrids of especially autotetraploid *L. purpureum* could scarcely be conceived to be the cause of the marked improvement in fertility often characteristic of these (BERNSTRÖM, 1954). For the corresponding diploid F_1 hybrids were characterized by perfectly good fertility, showing that there could be no structural chromosomal differences between the lines. The good fertility in *L. purpureo-bifidum* may rather be chiefly due to a heterotic effect of the kind indicated above. Without further experimental investigations of the question, however, this must remain an assumption.

For systematic reasons and on account of the appearance of the diploid hybrids, *L. purpureo-bifidum* was expected to agree at least closely with *L. hybridum* as regards appearance; this hope, however, was rather poorly fulfilled. But the main interest centres, as will soon appear from the crossing experiments between the species mentioned and *L. purpureo-bifidum*, round the morphological comparison with *L. hybridum*. *L. purpureo-bifidum* is in its appearance and in many details more reminiscent of normally grown F_1 hybrids between *L. hybridum* and tetraploid *L. purpureum* than of any of the pure species studied. It is about half again as tall as the cultivated lines of *L. hybridum*, and about twice as tall as *p*60 (*cf.* Fig. 20). The plants are

Fig. 20. To the left tetraploid *L. purpureum* (*p*60), in the centre *L. purpureo-bifidum*, to the right *L. hybridum* (*h*37).

more stiffly erect than those of *L. hybridum*, and they lack the wide branching characterizing fully grown individuals of this species. The leaves are drawn out to a more pointed triangular form than the roundedly cordate leaves of *L. hybridum*, and have more triangular indentations than have the leaves of the latter; they are, moreover, not so markedly double-indented as these. The flowers of *L. purpureo-bifidum* are quite as large as the biggest in *L. hybridum*, rather similar to these, but diverge especially in the slight indication in the upper lip of the two ears which are such a characteristic feature of the flowers of *L. bifidum*. The calyces of *L. purpureo-bifidum* have longer and more pointed sepals than one finds in *L. hybridum*. Summarizing, it may be said that several taxonomically important features clearly distinguish *L. purpureo-bifidum* from all the lines of *L. hybridum* studied, but it is more like the latter in appearance than any of the other species.

The morphological variation between the plants of *L. purpureo-bifidum* was very slight, as was also that in respect of pollen fertility and seed-setting. No detailed studies have been carried out on the possible occurrence of aneuploids or other chromosomal aberrations; if any such occur — as is likely — they are probably very few in number.

3. *L. hybridum*×*L. purpureo-bifidum*

a. Cytology

It has been possible to study the reduction division in three plants of *h*49×*L. purpureo-bifidum*. All the plants had $2n=36$. The investigations of the chromosome pairing in I M are summarized in Table 7. From this it emerges that 37 % of the cells had 2 to 4 univalents. The corresponding number in *L. purpureo-bifidum* was 5 % (Table 5). In *L. hybridum* 4 % of the cells had a couple of univalents, and no cell was observed with 4 univalents (BERNSTRÖM, 1953 b, p. 402). The difference in the occurrence of univalents as between the hybrids and *L. hybridum*

TABLE 7. *Frequencies of configurations and univalents in I M of hybrids between L. hybridum and L. purpureo-bifidum.*

Per cell	Plant No. 1	2	3	n
1 III + 16 II + 1 I		2		2
18 II	6	37	39	82
17 II + 2 I	5	17	18	40
16 II + 4 I		5	4	9

and *L. purpureo-bifidum*, respectively, was thus highly significant; it shows that there must have been chromosome-structural inhomologies between the two allotetraploids, unless the defective syndesis was caused by other factors having an unfavourable effect on the chromosome pairing; this, however, there is no reason to suspect.

As regards later stages of meiosis, I A appeared to proceed normally in 19 PMC's, though in many of them it was not possible to check the distribution of chromosomes. This was also impossible in II M; 75 PMC's seemed regular, while in 5 others one univalent lay in the cytoplasm outside the plates; and in one pollen mother cell it seemed as if a univalent had divided, one chromatid being situated in each plate, and none being oriented towards the poles.

b. Fertility and morphology

Hybrids have been produced between the respective lines *h*37 and *h*49, and *L. purpureo-bifidum*. The percentage of hybrid seed set was > 30. This was also the case after cross pollination of *h*89 with *L. pur-*

TABLE 8. *Pollen fertility and seed-setting in hybrids between L. hybridum and L. purpureo-bifidum, with their parent lines, in 1954.*

Hybrids, and parent lines	Pollen fertility		Seed-setting	
	$\bar{x} \pm m$	n	$\bar{x} \pm m$	n
*h*49 × *L. p.-b.*, F_1	93,3 ± 0,72	10	73,5 ± 0,95	16
*h*37 × *L. p.-b.*, F_2*	96,0 ± 0,81	9	77,6 ± 2,26	10
*h*37	99,4 ± 0,19	10	86,8 ± 2,40	10
*h*49	98,9 ± 0,35	10	91,7 ± 0,68	10
L. p.-b.	99,8 ± 0,05	20	59,4 ± 1,81	21

* Dwarfish plants are not included. *Cf.* the text.

pureo-bifidum. Of 44 seeds in this combination, however, only 6 had visible embryos. Three of these were too weak to develop, while the remaining three were pure *L. hybridum*. Thus, no hybrid plants could be obtained from *h*89 × *L. purpureo-bifidum*, despite apparently good crossability. Contemporaneously produced hybrid seeds of *h*49 × *L. purpureo-bifidum*, on the other hand, germinated well. What has been adduced above constitutes an illustration of the importance the selection of parent lines may have for the result of a species crossing. Other examples from *Lamium* have been adduced in an earlier paper (BERNSTRÖM, 1953 a).

The fertility in hybrids between *L. hybridum* and *L. purpureo-bifidum* is summarized in Table 8. The pollen fertility of the F_1 hybrids between *h*49 and *L. purpureo-bifidum* was quite significantly inferior to that of both parent lines ($P < 0{,}001$ for both comparisons). On account of predominantly closed flowering, the pollen fertility in the F_1 of *h*37 × *L. purpureo-bifidum* could be investigated only in a single plant; 200 pollen grains were all good. The pollen fertility in the F_2 generation, however, was significantly lower than that of the parents ($P < 0{,}001$), and the lack of empty grains in the only pollen sample from the F_1 was thus probably due to chance. No reduction of the pollen fertility whatsoever has characterized intraspecific hybrids of *L. hybridum*, though hybrids between precisely *h*49 and *h*37 were not, it is true, examined (BERNSTRÖM, 1953 b). It was therefore also to be expected that hybrids between *L. purpureo-bifidum* and different lines of *L. hybridum* should show approximately the same degree of fertility.

The seed-setting in the hybrids was roughly intermediate between that of the parents, and the effect upon the order of magnitude of the seed-

setting that may be exercised by the rather slight partial sterility as this is deduced from the quality of the pollen is probably inconsiderable as compared with the significance in this respect of the deficiencies in seed fertility inherited from *L. purpureo-bifidum*. We shall revert in the discussion to the import of the hybrids' partial sterility.

The two kinds of F_1 hybrids had the same appearance as the parents. They were not very large, attaining only to heights of between $^1/_4$ and a good $^3/_4$ of the tallness of these. The seasonal variations probably exercised an effect upon the relative size of the hybrids. Especially the hybrids with *h*49 showed considerable variations in size, which was probably due to a combined effect of poor vitality (*cf.* below) and sensitivity to changes in the environment. This sensitivity reminds one, though it was still more apparent, of that characteristic for *L. hybridum* (*cf.* BERNSTRÖM, 1953 b, p. 398). C_2 of *L. purpureo-bifidum*, of which the hybrids with *h*49 were the progeny, was already very uniform, and it is thus improbable that the variation in size as between the F_1 hybrids was conditioned by heredity.

Despite the small size of the plants, there were few unharmonious features in their appearance. Most apparent was a tendency, especially marked in the leaves of the stem, to bend downwards in a characteristic fashion, to roll up slightly, and to turn reddish. The flowering tended to be closed. The small size of the hybrid plants and characteristic abnormalities in the leaves testify to deficiencies in the cooperation of the parents' genotypes of a kind that has never been observed in intraspecific F_1 hybrids of *L. hybridum*.

Of 18 F_2 plants from *h*37 × *L. purpureo-bifidum*, 3 were fully developed, 4 were somewhat smaller, 3 were still smaller and, together with the 4 preceding ones, corresponded rather to the F_1 hybrids. Eight plants were miserably small dwarfs, in part not flowering. The boundaries between the three first-mentioned groups were not always distinct, and it was not possible to trace any correlation between size of plant and degree of sterility. We shall revert later to the segregation found.

4. *L. intermedium* × *L. purpureo-bifidum*

From cross pollination of altogether 146 flowers of *L. intermedium* with *L. purpureo-bifidum* one hybrid plant was obtained. Another plant died at an early stage; this was possibly also a hybrid.

MÜNTZING (1926), JÖRGENSEN (1927) and myself (BERNSTRÖM, 1953 a) have all failed to hybridize *L. intermedium* and *L. hybridum*; each of

us used his own experimental material, so that the incompatibility between the species may be considered to have been shown rather definitely. As it has now proved possible to hybridize *L. intermedium* and *L. purpureo-bifidum,* the difference in the compatibility with *L. intermedium* which has thus been shown between *L. hybridum* and *L. purpureo-bifidum* implies still another important genic difference between these latter.

The single hybrid plant obtained was in size, as well as in the appearance of the leaves, the flowers and the calyces, on the whole intermediate between the parents. It had a seed-setting of 39 %. All the branches were cut for fixation of the buds, though unfortunately it was not possible to study any meiotic divisions. As *L. intermedium* is an allotetraploid of *L. purpureum* and *L. amplexicaule* (*cf.* below), the extent of the bivalent formation in I M might on the whole have been a measure of the degree of homology between the genomes of *L. bifidum* and *L. amplexicaule*. To judge from the chromosome pairing of *L. hybridum* × *L. amplexicaule* and *L. hybridum* × *L. purpureo-bifidum*, respectively, a rather regular bivalent formation was to be expected; *cf.* the discussion below.

5. *L. purpureo-bifidum* × *tetraploid L. purpureum*

Reciprocal hybrids have been produced between *L. purpureo-bifidum* and the lines *p*60 and *p*61, respectively. The crossability was about the same in both directions, and equalled that between *L. hybridum* and tetraploid *L. purpureum* (*cf.* BERNSTRÖM, 1953 a). In both the combinations the plants were in height and vegetative development approximately intermediate between the parents, and thus normally developed. The hybrids with *p*60, *i. e.*, the line entering *L. purpureo-bifidum*, were, however, distinguished by frequently unsymmetrical shape of leaf and indentation, somewhat crumpled leaf-surface, and occasionally disproportionately small leaves in the inflorescence of the stem, all indications of a slight disharmony. Such features were almost entirely absent in the hybrids with *p*61.

No cytological examinations have been made.

The main interest in these hybrids is due to the circumstance that both kinds were of normal size, and that those with *p*61 were not dwarfs, as are the results of crossing with *L. hybridum*. Thus we have here still another genetic difference between this species and *L. purpureo-bifidum*; this will be discussed in greater detail below.

The less prominent disharmonious features in the hybrids with *p*61 show merely that one or more genes of *p*53 (*i. e.*, the diploid line of origin of *p*60) cannot co-operate without friction with the genome of *L. bifidum* subsp. *balcanicum* where *p*53 is over-represented. The more normal development of hybrids with *p*61 may possibly depend on a heterotic effect produced by the joint action of both *L. purpureum* genotypes included; interlinear F_1 hybrids of *L. purpureum* were often characterized by heterosis (BERNSTRÖM, 1953 b).

B. L. hybridum×L. amplexicaule

1. *The triploid hybrids*

In an earlier paper (BERNSTRÖM, 1952) analyses of the chromosome pairing in I M in hybrids between *L. hybridum* and different lines of *L. amplexicaule* have been given. The commonest type of pairing proved to be 9 II+9 I, followed by 8 II+11 I. A multivalent occasionally occurred, and the variation in the number of univalents per cell was rather considerable. The conclusions drawn were that *L. amplexicaule*'s genome paired with the one genome from *L. hybridum*, but that the homology was incomplete. It should be added that one must also reckon with the possibility that pairing may take place between the odd genome and the others too, especially that from *L. amplexicaule*.[1] Irregular pairing conditions and the occasional occurrence of cells with 10 II+7 I in the hybrids under consideration indicate this (*cf.* BERNSTRÖM, 1952, Table 7).

The morphology of the hybrids has been described in detail by MÜNTZING (1926). Here it need only be added that in their vegetative development the hybrids are clearly characterized by heterosis, as has also been verified arithmetically where it has been possible to make correct comparisons.

The hybrids are completely seed sterile. Of about 5000 flowers examined only one had an ovule whose appearance indicated that a fertilization had taken place. The pollen fertility was only 1 %, but this low figure can scarcely explain the almost complete absence of fertilized ovules, or seeds, especially as the stigmas in the small and generally closed flowers are, as a rule, found pressed against the anthers. This is possible, as in the cleistogamic flowers of *L. amplexicaule*, owing to

[1] A sparse heterogenetic pairing takes place in triploid hybrids between tetraploid *L. purpureum* and diploid *L. amplexicaule* (*cf.* below).

the fact that the style, which is longer than the filaments, is bent. In the experimental material only the diploid hybrids between *L. purpureum* and *L. bifidum* subsp. *balcanicum* are characterized by a seed sterility so complete, while, *e. g.*, the triploid hybrids between tetraploid *L. purpureum* and diploid *L. amplexicaule* may develop one seed per plant. The seed sterility in the hybrids between *L. hybridum* and *L. amplexicaule* is not, however, genic, as the seed-setting in the hexaploid hybrids shows (*cf.* below).

2. *Allohexaploid L. hybrido-amplexicaule*

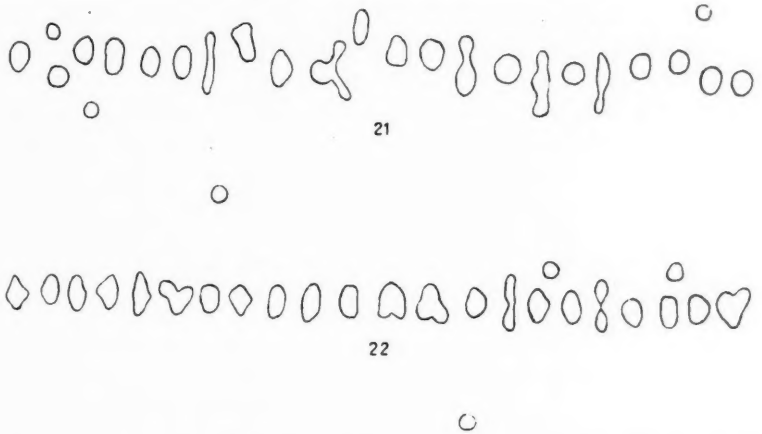
a. Cytology

The main purpose for which these hexaploids were produced was to cross them with diploid *L. amplexicaule*, in order thereby to compensate the failure of the combination *L. hybridum*×tetraploid *L. amplexicaule* (*cf.* BERNSTRÖM, 1953 a). Three different lines of *L. hybrido-amplexicaule* have been produced, in all cases through treatment with colchicine of triploid F_1 hybrids. The oldest line derives from hybrids between the lines *h*19 and *a*9, while the others comprise the lines *h*36×*a*33 and *h*36×*a*8. Most investigations, including the majority of crossing experiments (*loc. cit.*, and below), have been carried out on the first-mentioned of these; only where several lines are being spoken of at the same time is this called *L. hybrido-amplexicaule 1*, while the hexaploid of *h*36×*a*33 is denoted *L. hybrido-amplexicaule 2* and that between *h*36 and *a*8 *L. hybrido-amplexicaule 3*. Thus, if reference is made simply to *L. hybrido-amplexicaule*, it is the hexaploid of *h*19×*a*9 that is meant.

I M has been analysed in plants of *L. hybrido-amplexicaule 1* and *2*. Thanks to good fixations and a small number of multivalents, and through collation of the number of configurations+univalents per cell with the chromosome number of the respective plant — determined on somatic divisions —, it has, as a rule, been possible to analyse the pairing conditions. The analyses are given in Table 9. From this it appears that bivalents were by far the commonest configurations; the multivalents were on an average represented by about only one per cell, and there was about the same frequency of univalent pairs which did not owe their occurrence to trivalents. Despite the small number of cells it has been possible to analyse and the uncertainty from which the analysis must suffer, the evenness of the results as between the plants indicates that they do give a rather faithful picture of the pairing conditions among these hexaploids.

TABLE 9. *Frequencies of configurations and univalents at I M of L. hybrido-amplexicaule 1 and 2.*

2*n*	Line No.	Slide No.	Number of configurations and univalents per cell, and frequencies thereof															Means of conf's, *etc*, per cell		Number of cells
			IV's				1				1							0,3	0,0	
			III's	3		1	1		2	1	1							1,1	0,6	
			II's	19	25	24	22	26	23	25	23	27						23,9	24,9	
			I's	7	4	3	3	2	2	1	1							1,8	2,4	
54	*L. h.-a.* 1	1		1	1	—	2	1	3	5	4	2						Slide 1:	Slide 2:	19
	L. h.-a. 2	2		—	2	2	—	1	1	1	—	1								8
			IV's								1								0,1	
			III's	1		2	1	2	1										0,7	
			II's	21	24	21	23	22	24	26	24								23,5	
			I's	8	5	5	4	3	2	1	1								3,4	
53	*L. h.-a.* 2	3		1	2	1	1	1	2	2	1								Slide 3:	11
			IV's		1					1	1			1			1	0,3	0,2	
			III's	1	1		2	4	1	1	3		2	2	1			1,6	1,0	
			II's	22	20	24	21	18	23	21	18	25	22	20	24	26	24	21,3	23,2	
			I's	5	5	4	4	4	3	3	3	2	2	2	1			3,6	1,9	
52	*L. h.-a.* 1	4		1	1	1	3	1	2	1	1	—	—	—	1	—	—	Slide 4:	Slide 5:	12
	»	5		—	—	—	3	—	1	1	—	2	2	1	2	3	1			16



Figs. 21—22. I M in *L. hybrido-amplexicaule 1.* — Fig. 21, probably 1 IV+3 III+ +18 II+3 I; Fig. 22, 4 III+18 II+4 I. See the text. — ×2500.

Seen in polar view the I M-plates often show very pronounced »secondary associations». — Figs. 21 and 22 show the chromosomes from two cells of slide 4 in Table 9, with the configurations separately drawn. Fig. 21 must include 1 IV +3 III+18 II+3 I, but which are the trivalents is not clear. Fig. 22 shows 4 III+18 II+4 I; the trivalents are here clearly visible.

The predominant occurrence of bivalents must imply that the pairing is for the most part homogenetic. The two genomes from *L. hybridum* and *L. amplexicaule* which form heterogenetic bivalents in the triploid hybrids prove therewith not to be sufficiently homologous for a more extensive formation of multivalents to occur in the hexaploids. The maximum number of multivalents per cell would be nine, as against only one found; for comparison one may point to the hexaploids between *L. purpureum* and *L. amplexicaule* described below (p. 60), with four homologous genomes of *L. purpureum*. The average number of multivalents per cell was here nearly five. Thus in consideration also of the analyses of hybrids between, on the one hand, *L. hybridum* and tetraploid *L. purpureum*, and, on the other hand, tetraploid *L. purpureum* and diploid *L. amplexicaule* (*cf.* below), it may in all probability be regarded as established that *L. amplexicaule* does not enter in *L. hybridum*; we shall revert to this later.

b. Aneuploidy, fertility and morphology

Even if the investigation of the meiosis in *L. hybrido-amplexicaule 1* and *2* had not shown the occurrence of aneuploid plants, the multivalents and univalents in I M would nevertheless have led to the assumption of the occurrence of such plants, especially as a distinct variation in the shape of the leaves, height of the plants, earliness, fertility, *etc.*, characterizes the hexaploids. Investigations of *L. hybrido-amplexicaule 1* have shown, moreover, that aneuploid chromosome numbers are very common, are even, in fact, far more the rule than the exception.

Thus in C_4 the chromosome number in 39 plants selected at random varied between $2n=51$ and $2n=55$, and by far the commonest number was 52. The mother plants, three or four in number, to each of the generations (C_1)—C_4 had been chosen among the most seed-fertile plants in the next preceding generation, every generation having comprised between 19 and 63 individuals. In the progeny of six plants in C_4, of which two had $2n=54$, two had $2n=53$ and two had $2n=52$, and which were all the most seed-fertile within their respective chromosome classes, the chromosome number was determined for six to eight plants per progeny. In addition to this, C_1 was studied from two colchicine-treated triploid hybrids. Finally, some progenies in C_2, C_3 and C_6, likevise from plants with very good seed-setting in the respective generations, were investigated. Table 10 summarizes the results.

Especially if one assumes — as one may rather safely do — that the chromosome number in the branches of the triploid hybrids which were fertile after the colchicine treatment was $2n=54$, one may find from the table a consistent and very distinct feature in the progenies, *viz.*, the possession of lower chromosome numbers than those characterizing the respective mother plants. There is in the material an indication to the effect that the reduction in the progeny is less in proportion as the chromosome number is lower in the mother plant; but the material is too limited to justify any more definite statement in this connection. In combination with the observations made concerning the C_4 generation (*cf.* above) the data in Table 10 show, however, that *L. hybrido-amplexicaule* is not able to retain the original number $2n=54$.

It is uncertain, on the other hand, whether the line would be able to retain an average chromosome number of $2n=52$, or whether it would suffer a further loss of chromosomes if no selection for fertility was made. As has been pointed out above, the mother plants have so far always been among the best seed-fertile individuals in their generation.

TABLE 10. *Chromosome number variation and class frequencies in L. hybrido-amplexicaule 1* (*cf.* the text).

(In each offspring, the figure for the chromosome number of the mother plant is in italics.)

Year and progeny	Origin of mother plant	Number of chromosomes of mother plant	C-generation	Somatic chromosome numbers										n	x
				44	48	50	51	52	53	54	55	56	57		
1947															
1	—	—	C_1					2	2	4				8	53,3
2	—	—	C_1					4	3	—	—	1		8	52,9
3	prog. 4—46	54	C_5				2	1	3	(*0*)				6	52,2
4	»	54	C_5					3	2	*1*				6	52,7
5	»	53	C_5	1	—	—	2	3	*1*					7	50,7
6	prog. 1—46	53	C_5				1	1	*3*	1				6	52,7
7	»	52	C_5			1	1	*6*						8	51,6
8	prog. 4—46	52	C_5				1	*6*						7	51,9
1948															
1	prog. 1—47	54	C_2					3	6	*4*	5	2		20	53,9
1949															
1	prog. 1—48	55	C_3				1	4	—	3	*1*	2	1	12	53,8
2	prog. 3—47	51	C_6			10	*3*	1						14	50,4
3	prog. 8—47	51	C_6		1	3	*2*	—	2					8	50,8

TABLE 11. *Correlations between chromosome numbers and different characteristics in L. hybrido-amplexicaule 1* (for explanation, see the text).

Characteristics, and years	2n = 44		2n = 48		2n = 50		2n = 51		2n = 52		2n = 53		2n = 54		2n = 55		2n = 56		2n = 57	
	x ± m	n	x ± m	n	x ± m	n	x̄ ± m	n	x̄ ± m	n	x̄ ± m	n	x̄ ± m	n	x ± m	n	x̄ ± m	n	x ± m	n
1946																				
Seed-setting							16,2 ± 2,70	5	28,4 ± 2,51	21	27,3 ± 4,81	9	33,2 ± 5,61	3	25,9	1				
Florescence time							7,6 ± 1,75	5	8,8 ± 0,59	21	8,0 ± 0,71	9	6,0 ± 2,65	3	7	1				
1947																				
Seed-setting	10	1	—	—	16	1	20,1 ± 4,69	7	26,9 ± 1,54	26	29,9 ± 4,26	13	30,3 ± 3,38	6	—	—	6	1		
Indentations per leaf	12,3	1	—	—	11,0	1	13,3 ± 2,29	6	16,3 ± 0,57	23	16,4 ± 0,82	13	19,8 ± 0,63	5	—	—	—	—		
Florescence time	10	1	—	—	17	1	13,1 ± 1,86	7	8,3 ± 0,91	24	9,2 ± 1,87	13	4,5 ± 1,63	6	—	—	7	1		
1948																				
Seed-setting									30,8 ± 5,27	3	27,2 ± 2,66	6	20,1 ± 7,80	4	26,8 ± 3,46	5	24,6 ± 1,25	2		
Indentations per leaf									19,3 ± 0,66	3	18,1 ± 0,82	6	16,9 ± 0,36	4	18,7 ± 1,03	5	16,0 ± 0,50	2		
1949																				
Seed-setting			8	1	10,6 ± 3,00	13	24,1 ± 8,34	6	27,7 ± 8,39	5	26,4 ± 15,33	2	38,3 ± 0,60	3	25	1	34,5 ± 15,54	2	36	1
Indentations per leaf			—	—	11,8 ± 0,81	8	13,1 ± 1,23	4	18,5 ± 2,00	2	—	—	18,3 ± 1,08	3	—	—	19,0	1	17,0	1

This has probably helped to maintain the chromosome number, since the seed-setting is better in plants with higher than with lower chromosome number; *cf.* Table 11. As for the further vicissitudes of the chromosome number, this is a matter of the conditions obtaining in connection with a continued cultivation, and it is an open question what course the development of the chromosome number would take if the hexaploid were left to its own resources and proved to be able to survive in natural conditions. The chromosome number might then be expected to become the increasingly dominant feature, which was based upon definite chromosomes, possibly newly created through heterogenetic pairing, and which best assured the competitive ability of the allopolyploid in the environment most favourable to it.

The number of plants in Table 11, covering the same plant material as Table 10, are rather small for assessments of the influence of the chromosome numbers upon different properties. The risks of erroneous conclusions are in this material even greater than among autotetraploids; *cf.* BERNSTRÖM, 1954. The values in Table 11 may only be used for a study of the general tendencies, and not for detailed comparisons between classes of chromosome numbers. The values from 1948 clearly constitute a good illustration of the risk entailed in investigating only a few individual plant progenies, even after well fertile euploids, for on account of the contrary behaviour of its progeny as compared with the material from the other years in respect of the two tabulated properties, the mother plant to the 1948 generation may be assumed to have been a »compensated» or otherwise chromosomally unbalanced euploid plant. The properties investigated in *L. hybrido-amplexicaule* are the same as those in the above work; *cf.* that paper. The scope of the remaining experimental material has not allowed of a study of all properties in all the years.

As emerges from Table 11, the aneuploid chromosome numbers do not seem to have any markedly negative effect upon the seed-setting, though the tendency to decreasing fertility with increasing aberration of the chromosome numbers is apparent. The number of leaf indentations is in a corresponding way correlated with the chromosome number, and the same probably applies also to the time for the commencement of flowering, the euploid plants beginning to flower on an average some days before the aneuploids. In their property of having a greater number of leaf indentations than the aneuploid plants the euploid plants agree with both *L. intermedium* and tetraploid *L. amplexicaule* (BERNSTRÖM, 1953 b, 1954). The phenomenon thus gives the

impression of being more a sign of weakened power of growth owing to aneuploidy than merely a morphological result of a gene-deficiency. The number of leaf indentations has not so much to do with the actual shape of the leaf, concerning which more below.

Besides the properties tabulated in Table 11, the height of the plant in all the generations referred to in the table and the pollen fertility in 1949 were investigated. In none of the generations was it possible to trace any effect upon the height of the plant due to a change in the chromosome number. Its height is a poor measure of the vigour of a plant, which it was the aim of the investigation to ascertain, and it is therefore not impossible, rather, in fact, is it probable that changes in the chromosome number nevertheless affect the vegetative development. On the other hand, it is evident that in this case the effect in question must be rather slight. — The average pollen fertility was 84,2, and this, too, gave no evidence of any effect from changes in the chromosome number.

In its variation in chromosome number and the effect of this upon different properties *L. hybrido-amplexicaule* behaves in part otherwise than the autotetraploids of both *L. amplexicaule* and *L. purpureum* (*cf.* BERNSTRÖM, 1954). In the latter hypoploidy leads to a greater reduction of the seed fertility than in the former, and a diminution in the height of the plant and in the pollen fertility is then also, as a rule, traceable, as well as changes of a kind resembling those recorded in Table 11. As regards the variation in the chromosome number, hypertetraploid individuals in *L. purpureum* and *L. amplexicaule* are far commoner than hypotetraploid ones; and of the latter, plants with two chromosomes fewer than the normal are very uncommon, and have not been met with spontaneously. Further, and this is the most important point, progenies after hypotetraploids manifest a tendency to restore the original number, in clear contrast to what is observed in *L. hybrido-amplexicaule*.

Among the autotetraploids of *L. amplexicaule* and *L. purpureum* trivalents and quadrivalents occurred in rather large numbers, or about six out of nine possible, whereas there occurred scarcely one univalent, on an average, per cell. In *L. hybrido-amplexicaule 1* and *2* there was about one multivalent per cell, while the univalents were on an average two to three in number. Among the autotetraploids probably both unequal segregation of multivalents, and random distribution and loss of univalents contribute to irregular chromosome numbers in the gametes; in *L. hybrido-amplexicaule* the former factor may, on the other hand,

be expected to be of lesser, but the latter of somewhat greater, importance. Gametes with approximately the same numerical lack of chromosomes may be expected to occur in both categories; among gametes from the hypotetraploids of the pure species there are perhaps even deficits in relatively greater measure. In these circumstances the evident tendency in *L. hybrido-amplexicaule* successively to reduce its chromosome number, as compared especially with the marked re-establishment of the normal chromosome number in progenies after hypotetraploids, must be assumed to be due to a greater insensitivity in the gametes above all to chromosome deficiencies.

That forms with high chromosome numbers may more readily bear aneuploidy than those with a smaller number of chromosomes is a well-known fact from many plant genera, and is to be explained by the extensive duplications of the genes. In *L. hybrido-amplexicaule* the presence of four presumably rather related genomes may constitute one circumstance that prevents the deficient chromosome numbers from causing stronger gametic lethality, and from giving rise in various ways to marked setbacks among the hypohexaploid plants, and that also makes possible a real reduction of the original chromosome number. The question of the magnitude of this reduction has already been touched upon; in view of the regularity with which it occurs, and the fact that fertile and vigorous plants have occurred with $2n=44$ and 48, it appears likely that considerable reductions would be possible in protected conditions. Theoretically, and with a very large experimental material, it is of course conceivable that something resembling *L. hybridum* with 36 chromosomes might appear again, although probably modified as a result of heterogenetic pairing. The 36-chromosome counterpart, formed by the *L. amplexicaule* genome in likewise modified form and by the unpaired genome from *L. hybridum* in the triploid hybrids, might then perhaps also be produceable. But to try in this way to investigate the nature of *L. hybridum* — mainly on the hypothesis that the last-mentioned genome is identical with that of *L. purpureum* and that the product would thus be as nearly as possible *L. intermedium* — would be an uncertain undertaking and one requiring a great deal of work.

L. hybrido-amplexicaule 1, *2* and *3* resemble each other rather closely and are distinguished from the triploid hybrids in their darker green colour, coarser structure of the different organs, *etc.* In the summertime a number of the flowers are chasmogamic, large and well-developed, while others — the majority — are small and cleistogamic. During the

flowering in the autumn this type occurs almost exclusively. The very evident variability in different respects which characterizes *L. hybrido-amplexicaule* is manifested not least in the mode of flowering, the frequency of chasmogamic flowers in the summer varying rather considerably as between the individuals. Many appear to flower exclusively cleistogamically. In the respects referred to *L. hybrido-amplexicaule* is reminiscent of tetraploid *L. amplexicaule* (*cf.* BERNSTRÖM, 1954).

The variability is manifested in the shape of the leaves still better than in the mode of flowering. The appearance most typical for the line reproduces in a simplified and coarser form the appearance of the leaves found on the triploid hybrids. From this appearance there occur finer or coarser deviations over a scarcely surveyable range, the deviations being shown in the number of indentations, their shape and size, the extent of the double indentation, the size of the leaves, their proportions and degree of division into lobes, *etc.* Some specimens show features which remind one of the leaf shape of *L. amplexicaule*, while others, on the other hand, underline features of *L. hybridum*. These manifold manifestations are probably due both to differences in the chromosome number and to gene recombinations after heterogenetic pairing. Attempts to trace some outstanding shapes of leaf of single plants in their progeny have not, however, led to any positive results. Attempts to assess the approximate chromosome number from the actual appearance of the leaves, and not merely from the number of indentations, have likewise failed.

3. *L. hybrido-amplexicaule*×*L. intermedium*

a. Cytology

Of three hybrid plants between *L. hybrido-amplexicaule 1* and *i*58, two had $2n=42$ and one had $2n=43$.

It has been possible to study the chromosome pairing in I M in one plant of each chromosome number. As the fixations were good and the somatic chromosome numbers known, it was possible in the cells referred to in Table 12 to determine the nature of the configurations with rather considerable assurance. A few mistakes are of course not out of the question.

From the investigations of the chromosome pairing in the hybrids earlier described it has emerged that *L. hybridum* has two kinds of genomes, one of which is on the whole homologous with those of

TABLE 12. *Frequencies of configurations and univalents at I M in hybrids between L. hybrido-amplexicaule 1 and L. intermedium.*

2n	Slide No.	Number of configurations and univalents per cell, and frequencies thereof																			Means of associations per cell	Number of cells
		IV's								1	1						1		1		0,2	
		III's	3	1	3		2	1	3	1		2	4	1	3	2	1	3	3	4	2,0	
		II's	11	15	12	17	14	16	13	14	16	15	12	17	14	16	16	15	13	14	14,7	
		I's	12	10	10	9	9	8	8	8	7	7	7	6	6	5	4	4	4	3	6,8	
43	1		1	1	1	1	1	3	1	1	1	1	1	1	2	1	2	2	1	1		23
		IV's					1			1		1									0,2	
		III's		1		2	2	1	2	1	3	3									1,4	
		II's	17	16	18	15	13	17	16	16	15	13									15,8	
		I's	8	7	6	6	6	5	4	3	3	3									5,6	
42	2		1	6	2	3	1	1	3	1	1	1										20

L. purpureum, and the other in all probability partially homologous with those of *L. amplexicaule*. Further, one kind of genome in *L. intermedium* is homologous with those of *L. amplexicaule*; and the other with *L. purpureum*'s. As will be shown below, *L. intermedium* is as a matter of fact an allotetraploid of these two species. In hybrids between *L. hybrido-amplexicaule* and *L. intermedium* one may therefore expect bivalent pairing between the *L. purpureum* chromosomes from the latter and their homologous from *L. hybridum*, and bivalent and trivalent formation between the others. This means 18 configurations as well as a number of univalents, which do not, as a rule, exceed nine in number.

From the survey in Table 12 it is apparent that the figures actually found agree on the whole with those expected. The few quadrivalents may be assumed to be due chiefly to the same structural differences between the genomes as caused the occurrence of multivalents in the triploid hybrids between *L. hybridum* and *L. amplexicaule* (*cf.* Table 7, BERNSTRÖM, 1952, and above, p. 40). The lower average numbers of configurations per cell than expected (16,9 for slide 1 and 17,4 for slide 2) are probably chiefly due to the absence of 2 or 3 chromosomes. In all likelihood these belonged to the *L. amplexicaule* genome or to the genome homologous herewith, according to what is indicated by the observations on *L. hybrido-amplexicaule*. If the missing chromosomes belonged to *L. amplexicaule*, the homology between the corresponding chromosomes from *L. intermedium* and the chromosomes from *L. hybridum* which are most closely homologous with these may have been too deficient to make possible a regular pairing. It is very probable, too, that structural differences also exist between the two real *L. amplexicaule* genomes entering the F_1 hybrids, to judge from the general occurrence of such differences in *L. amplexicaule* (BERNSTRÖM, 1952). This may further contribute to a picture of the pairing deviating from what might be expected.

b. Fertility and morphology

Of the cytologically analysed plants above, that with 43 chromosomes had a seed-setting of 2 % and a pollen fertility of 60 %. The corresponding values in the plant with 42 chromosomes were 9 % and 90 %. The somewhat greater frequency of bivalents and the lower frequency of univalents in combination with the somewhat lower chromosome number may possibly have caused the better fertility in the latter plant, through a less varying chromosome number in the gametes, and a relatively larger number with ±diploid chromosome numbers.

The fertility in hybrids between *L. hybrido-amplexicaule 3* and *i*39 as well as the parent lines was:

	Seed-setting	n	Pollen fertility	n
L. hybrido-amplexicaule 3 (C_3) ..	43,8	9	91,1	8
*i*39	71,8	14	98,7	13
F_1 hybrids	30,4	10	89,0	10

The relatively high fertility in these hybrids, which have not been cytologically investigated, together with the cytological observations from the hybrids studied and from hybrids between *L. hybridum* and *L. amplexicaule*, permits of the assumption that tetraploid hybrids between *L. hybridum* and *L. intermedium*, if it had been possible to produce these, would have been characterized by a predominantly bivalent pairing and by fairly good fertility.

In their general appearance the hybrids were considerably more reminiscent of *L. intermedium* than of *L. hybrido-amplexicaule 1* and *3*. The shape of leaf and the appearance of the calyces and the flowers, however, were intermediate. The flowers were as large as those of *L. intermedium*, with just as little tendency to reduction in size and to cleistogamy as they, in contradistinction to those of *L. hybrido-amplexicaule*. This is somewhat surprising; considering the genomes entering in the hybrids and the types of flower characterizing the different combinations of the same, one would rather have expected a strong tendency to cleistogamy. The marked chasmogamy of the F_1 hybrids indicates the existence of genes with an unequal degree of dominance for chasmogamic flowering in the *L. purpureum* genome entering *L. intermedium*, and the genome of *L. hybridum* homologous with this.

4. *L. hybrido-amplexicaule*×*tetraploid L. amplexicaule*

a. Cytology

The best slides with reduction divisions that it was possible to produce derived from two plants of *L. hybrido-amplexicaule 1*×*a*15, each with $2n=44$; I M was the only stage of division occurring and the PMC's were few, obviously because the analysed buds would have become cleistogamic; nor was it possible to determine the nature of the configurations. The results are summarized in Table 13.

On account of the presence of three homologous, one partly homologous and one odd genome at least nine univalents, on an average, per cell might be expected, and likewise at least nine and probably more

TABLE 13. *Chromosome pairing at I M in hybrids between L. hybrido-amplexicaule and tetraploid L. amplexicaule; 2n=44.*

Slide No.	Number and frequencies of configurations and univalents per cell															Number of cells
	Conf's	12	10	13	11	12	13	12	11	10	15	13	12	11	13	11
	I's	14	14	13	13	12	11	11	11	11	10	10	10	10	9	9
1		1	—	1	1	—	1	1	1	1	—	—	1	1	1	1	11
2		—	1	—	—	1	—	—	1	—	1	2	—	—	1	—	7

configurations, predominantly multivalents. This obviously agrees, moreover, with actual experience. Owing to the rather large numbers of univalents, however, the combinations of the different configurations which may be concealed behind the numbers of univalents+configurations per cell are rather numerous, which renders impossible an even approximate calculation of the actual pairing conditions. From the cells with 11 I+10 configurations, 9 I+11 configurations and 10 I+11 configurations it appears that 3, 2 and 1 quadrivalents, respectively, must have occurred. This shows the occurrence of the heterogenetic pairing that must of course be expected in the light of experience from the different above-described hybrid combinations, and that is probably rather extensive, since the genome from *L. hybridum* partly homologous with those of *L. amplexicaule* is odd.

An F_2 generation after the above-analysed plants, comprising only 11 individuals, had an average chromosome number of 42,7; the extremes were $2n=39$ and $2n=46$. The reduction in the number of chromosomes may be assumed to have been due to the elimination in the course of meiosis of the chromosomes of the unpaired genome, which derived from *L. hybridum*.

b. Fertility and morphology

Owing to the fact that the flowering is almost exclusively cleistogamic in the hybrids, it was not possible to investigate the pollen fertility. The seed fertility in the hybrids between *L. hybrido-amplexicaule 1* and the tetraploid *L. amplexicaule* lines *a*15, *a*14 and *a*55 was 3,3 % (n=9), 3,5 % (n=9) and 5,4 % (n=4), respectively. The F_1 hybrids with *a*15, which is the tetraploid to the line *a*9 of *L. amplexicaule* entering in *L. hybrido-amplexicaule 1*, thus had no better fertility than the others. *a*14 corresponds to the diploid *a*8, and the hybrids between *a*8 and *a*9 are characterized by only about 70 % seed fertility (BERNSTRÖM, 1952). Thus, this partial sterility left no trace at all in the pentaploid hybrids;

anything of this kind was scarcely to be expected, moreover, by the side of the far more powerful causes of sterility existing in the irregular meoisis of the pentaploids, and the presence of three different kinds of genomes.

The hybrids between *L. hybrido-amplexicaule* and tetraploid *L. amplexicaule* are on the whole intermediate in appearance between the parents, tending more to the former than to the latter.

C. Tetraploid L. purpureum × diploid L. amplexicaule

1. *The triploid hybrids*

a. Cytology

The somatic chromosome number has been found to be $2n=27$ in plants from three different line combinations.

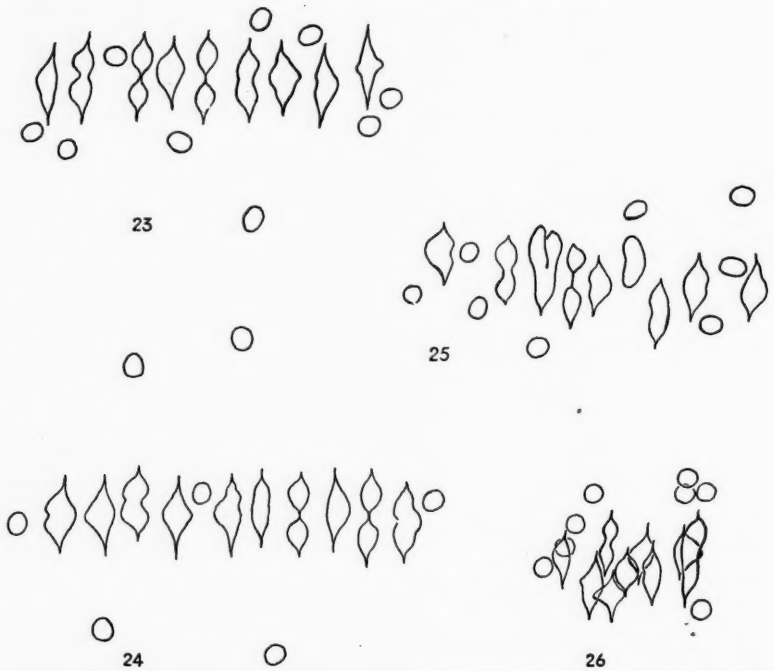
The chromosome pairing in I M has been analysed in hybrids between *p*7 and *a*8; no other stages of meiosis have been studied. The fixations were unusually good. The results are summarized in Table 14.

TABLE 14. *Frequencies of configurations and univalents at I M in hybrids between tetraploid L. purpureum and diploid L. amplexicaule.*

Configurations and univalents per cell	Slide No.					n
	1	2	3	4	5	
1 III + 8 II + 8 I	2		4	1		7
1 III + 7 II + 10 I				1		1
10 II + 7 I	4		3	3		10
9 II + 9 I	100	42	51	170	150	513
8 II + 11 I	4	2		4	1	11
Total	110	44	58	179	151	542

About 95 % of the PMC's thus had 9 II+9 I. In only 1,5 % of the cells did a trivalent occur. The majority of the trivalents were rod-shaped, but one seemed to be Y-shaped, thus this one, too, with two chiasmata. Also the number of cells with 10 II+7 I and 8 II+11 I was small. — Figs. 23—26 show some of the cells examined.

The pairing conditions found show that the homology between the genomes of *L. purpureum* and *L. amplexicaule* must be very slight, and that the bivalent pairing must in the great majority of cases be homogenetic. The few trivalents imply, however, that at least one *L. am-*



Figs. 23—26. I M in tetraploid *L. purpureum*×diploid *L. amplexicaule*. — Fig. 23, 9 II+9 I; Fig. 24, 10 II+7 I; Fig. 25, 1 III+8 II+8 I; Fig. 26, 1 III+8 II+8 I. The chromosomes are separately drawn in Figs. 23—25, with their mutual levels retained. — ×3000.

plexicaule chromosome is in part homologous with a chromosome from *L. purpureum*; through the occurrence of trivalents it also appears probable that some of the cells with 9 II+9 I had a bivalent through heterogenetic pairing. Cells with 10 II+7 I may indicate heterogenetic association in two bivalents simultaneously, which would imply partial homology between two of the chromosomes from each of the genomes. The extra bivalent may also conceivably be due to an association between two *L. amplexicaule* chromosomes compelled by an emergency which would appear to be comparable with that in haploid plants, and in this case it will be due either to partial homology between the chromosomes or to purely chromosome-mechanical factors (LEVAN, 1942, 1945). — The last-mentioned explanation is then of course also conceiv-

able for, *e. g.*, the cells with 6 I+9 configurations in the aneuploid plant of *L. intermedium*×tetraploid *L. purpureum* (*cf.* Table 1).

b. Fertility and morphology

In a previous paper some instances have been given of the considerable difference in the ease with which hybrid seeds may be produced between tetraploid *L. purpureum* and diploid *L. amplexicaule*; the result may be due to the choice of line in respect of either the one or the other of the parent species (BERNSTRÖM, 1953 a). Out of 14 tested crossing combinations nine have resulted in hybrid plants, and among these nine that between *p*7 and *a*8 has been chiefly studied. The hybrids in all the combinations have been vigorous and with the same general appearance, although each in its way very clearly stamped with the properties of the parent lines; thus as regards height, shape of leaf, type of leaf indentation, appearance of the flowers, *etc.*

The pollen fertility in *p*7×*a*8 has varied between about 5 % and about 15 % in different generations. The pollen grains are very uneven as to size and the filled grains varying in point of stainability, so that a morphological classification is rather difficult.

Relevant to the question of the seed fertility is the morphology of the flowers. They are chasmogamic, except in the late autumn, when they do not open. They are more faintly coloured than the flowers of the parent species, and not even so large as the flowers of diploid *L. purpureum*, features which are obviously connected with their high degree of sterility (*cf.* below, and p. 90). In appearance they are most like the flowers of *L. purpureum*, and are entirely without the long tube of *L. amplexicaule*. The anthers dehisce but the style is generally longer than the stamens, rendering self-fertilization impossible, and the coordination of stamens and pistil is also defective in other ways, which is common in species hybrids in the genus.

In the autumn of 1945 the flowers of seven plants of *p*7×*a*8 were self-pollinated daily by hand. The result was on an average one mature seed per plant; five times as many apparently maturing ovules were not able to ripen, or lacked visible embryos when husked later on, although the husk of the seed had matured. Hybrids of the same or other line combinations, that in other years had been placed either in greenhouses isolated from insects or out-of-doors in frames in order if possible to become self-pollinated through insects, set only a few scattered seeds, which were unable, for the rest, to germinate, with one important ex-

Fig. 27. To the left diploid *L. amplexicaule* (*a*8), to the right tetraploid *L. purpureum* (*p*7), in the centre their triploid F_1 hybrid.

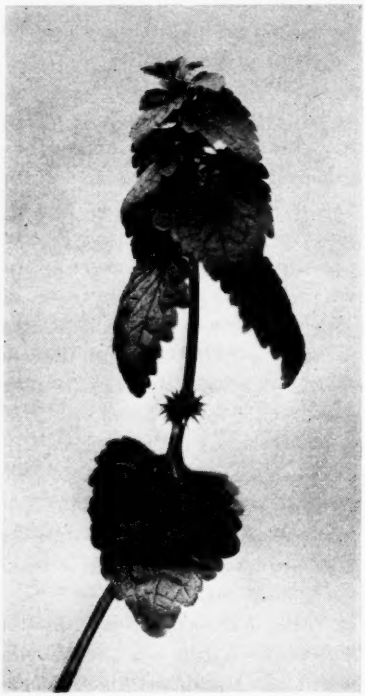


Fig. 28. Inflorescence of triploid F_1 hybrid between tetraploid *L. purpureum* and diploid *L. amplexicaule* (=*p*7×*a*8).

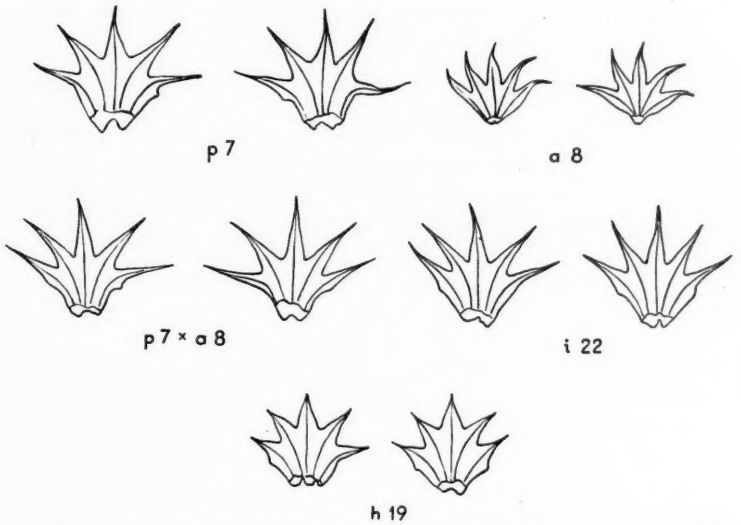


Fig. 29. Opened calyces from tetraploid *L. purpureum* (*p*7), diploid *L. amplexicaule* (*a*8), the F_1 hybrid between them (*p*7×*a*8), *L. intermedium* (*i*22), and *L. hybridum* (*h*19). — ×1,8.

ception (*cf.* p. 77). The incapacity for regular self-fertilization must have been a contributory cause of this sterility.

In their appearance the hybrids are most reminiscent of diploid *L. purpureum*, but the branches are longer than in the latter in relation to the height of the stem; and similarly, the internodes in the inflorescence are longer in the hybrids, both features from *L. amplexicaule*. The leaves are more rounded than in *L. purpureum*, with larger and in part also double indentations, and frequently with a tendency to a trilobate form of leaf. Fig. 27 shows a plant of *p*7×*a*8 between the parents, and Fig. 28 a close-up of a plant of the same combination but from another generation.

In contradistinction to the leaves, the calyces of *Lamium* have as regards their appearance always proved to be rather little sensitive to variations in the chromosome number; every species, moreover, has its own typical appearance in respect of the calyx. The calyces of the hybrids between tetraploid *L. purpureum* and diploid *L. amplexicaule* do not resemble those in either of the parent species; they are, on the other hand, remarkably like those of *L. intermedium*, but not at all like those of *L. hybridum*. Only in the circumstance that in the hybrids the

sepals are somewhat narrower and more pointed than in *L. intermedium* is it possible to distinguish the calyces through their appearance. The calyces of all four species and of the hybrids under discussion are reproduced in Fig. 29, where the calyces of the hybrids seem to have an appearance which is on the whole intermediate between those of *L. purpureum* and *L. intermedium*. In reality, however, and when seen in larger numbers on the plants, they remind one much more of *L. intermedium* than of *L. purpureum*.

2. *Allohexaploid L. purpureo-amplexicaule*

a. Cytology

L. purpureo-amplexicaule 1 has been produced through colchicine treatment of triploid hybrids between *p*7 and *a*8, while *L. purpureo-amplexicaule* 2 derives in the same way from *p*42×*a*13. The intention with the hexaploids was to try to hybridize them with in the first place diploid *L. amplexicaule*, to attempt in this way a direct production of *L. intermedium*. If this did not prove successful, the hexaploids might instead be conceived as hybridized with tetraploid *L. amplexicaule*, and the odd *L. amplexicaule* genome be eliminated after some generations' selection, resulting in tetraploids equivalent to *L. intermedium*. Neither of the projects succeeded, as emerges from an earlier paper (BERNSTRÖM, 1953 a, Table 1); since this was written, a further 114 flowers of *L. purpureo-amplexicaule* have been vainly crossed with diploid *L. amplexicaule* in another line combination than that first tried.

No particular interest in connection with the investigations on the relationships between the species attaches to the cytology of the hexaploids. A trial fixation of buds from the two lines produced proved, however, good enough for a rather reliable analysis of some few cells in I M from a plant of each line. The analyses were facilitated by the fact that the somatic chromosome numbers of the respective plants were also known. The results are reproduced in Table 15.

With a knowledge of the chromosome pairing in I M in the triploid hybrids one must in the hexaploids expect practically pure homogenetic pairing. It would then be of interest to compare the observed number of multivalents per cell, in all $4_{,8}$ and $4_{,7}$, with those in autotetraploid *L. purpureum*, and, especially, with those in *L. hybrido-amplexicaule*. In the former the corresponding figure was almost 6, the multivalents chiefly being quadrivalents (BERNSTRÖM, 1954). The lower total and the greater proportion of trivalents in *L. purpureo-amplexicaule* may,

TABLE 15. *Frequencies of configurations and univalents at I M in L. purpureo-amplexicaule 1 and 2.*

Line No.	2*n*	Number of configurations and univalents per cell, and frequencies thereof											Means of conf's, *etc.*, per cell	Number of cells
		IV's	2	3	2	3	4	5	4	3	3	4	5	3,4	
		III's	3	3	2	2	1	1	2	1	0	0	0	1,4	
		II's	16	14	18	16	16	14	15	19	21	19	17	16,9	
		I's	5	5	4	4	3	3	2	1	0	0	0	2,5	
L. p.-a. 1	54		1	1	1	2	1	1	1	2	1	1	1		13
		IV's	2	4	2	4	1	4						2,7	
		III's	2	2	1	3	3	1						2,0	
		II's	19	15	21	14	21	18						18,1	
		I's	3	3	2	2	0	0						1,9	
L. p.-a. 2	55		2	1	1	1	1	1							7

however, as well be due to the limited material for comparison as to a reduction of the chiasma frequencies in consequence of the higher chromosome number.

In *L. hybrido-amplexicaule 1* and *2*, of which slides Nos. 2 and 3 from the latter were fixed at the same time as that of *L. purpureo-amplexicaule 1* (Table 15), the number of multivalents per cell is only about one third or one fourth of that in *L. purpureo-amplexicaule*. As autotetraploid *L. amplexicaule* has proved to have the same multivalent frequency as *L. purpureum* (BERNSTRÖM, 1954), the difference in the pairing conditions between the two kinds of allohexaploids may with great probability be taken as a further confirmation of the conclusion stated earlier to the effect that the low multivalent frequency in *L. hybrido-amplexicaule* is due to extensive structural differences between the two genomes which associate in the triploids.

To judge from a few PMC's in I A, II M and II A in the slides included in Table 15, as well as in a couple of others, the reduction divisions appeared to proceed without other disturbances than a few lagging univalents. From the above investigations it appears obvious that gametes with varying chromosome numbers must be formed; preliminary investigations on somatic chromosome numbers have shown, moreover, that aneuploids are common, but no extensive studies have yet been carried out.

b. Fertility and morphology

The fertility is low in both lines, as appears from Table 16. The variation between the plants is, however, rather great as regards the degree of seed-setting; the best plants in this respect had in C_2 of *L. purpureo-amplexicaule 1* 17 and 19 % seed-setting, respectively. The chromosome numbers in these was observed to be $2n=54$ and 52. Several other plants approached them as regards the degree of seed-setting, while others seemed to be practically sterile.

TABLE 16. *Pollen fertility and seed-setting in L. purpureo-amplexicaule 1 and 2.*

Years and lines	Number of plant progenies	C-generation	Mean of seed-setting	Number of plants	Mean of pollen fertility	Number of plants
1948						
L. p.-a. 1	8	C_1	4,9	19	59,8	11
L. p.-a. 2	7	C_1	1,9	16	51,7	16
1949						
L. p.-a. 1	5	C_2	6,8	46	61,1	41
L. p.-a. 2	6	C_2	0,6	41	60,4	40

The seed-setting is astonishingly low as compared with what it is in, *e. g.*, *L. hybrido-amplexicaule*, *L. intermedium* and tetraploid *L. purpureum*. This is, however, probably far from an expression for the actual seed-setting capacity of the lines, and this for two reasons. The one is that the anthers more frequently remain closed than they dehisce, and this is probably connected with the fact that they contain small amounts of pollen. The other reason is that in the large and unusually wide flowers characterizing both lines the co-ordination of stamens and pistil for autogamy is often very poor.

Both lines are characterized by the low height of plants, as a rule sparse branching and considerable size of various organs, above all the calyces and the flowers. *L. purpureo-amplexicaule 1*, however, has far smaller leaves than *L. purpureo-amplexicaule* 2, with a frequently disharmonious appearance, especially conspicuous in the bowl-shaped upturned edges of the leaves and the crumpled leaf surface. In other features, too, the former shows signs of physiological disharmony, and it is also considerably weaker, less branching and shorter. The two kinds of triploid hybrid were of equal size. Between the plants in the two

hexaploid lines, but especially in *L. purpureo-amplexicaule 1*, the variation in size and development is rather considerable. — To the extreme left in Fig. 36 (p. 75) may be seen a normal plant of *L. purpureo-amplexicaule 2*. The two plants to the right in the picture represent the parent lines.

In the weaker growth, the small and bowl-shaped leaves and other aspects of their appearance the plants of *L. purpureo-amplexicaule 1* are often very reminiscent of the feeble hybrids between *L. intermedium* and tetraploid *L. purpureum*. Both have it more or less as a feature in common that *p*7 is the *L. purpureum* component, and that this species is more represented in the genotype than *L. amplexicaule* (2 : 1 and 3 : 1, respectively). These circumstances support the alternative assumption mentioned above (p. 8) concerning the cause of the disharmonious development of the majority of the hybrids between *L. intermedium* and *p*7, *viz.*, that the particular genotype of the latter is to blame, and that thus other lines of tetraploid *L. purpureum*, such as *p*30 and *p*42, may produce vigorous hybrids with *L. intermedium*.

3. *L. purpureo-amplexicaule* × *L. hybrido-amplexicaule*

Three plants have been raised in this cross combination, one of them after reciprocal crossing. In their height and appearance, as also in the finer features, they occupied on the whole an intermediate position. The seed-setting was 3, 6 and 44 %, respectively. The second plant showed 77 % pollen fertility. No cytological examinations were carried out on these hybrids. The most interesting thing about them is that it is possible to produce them, for from the viewpoint of their genomes they are about equivalent to hybrids between octoploid *L. intermedium* and tetraploid *L. hybridum*.

III. Investigations on some F_2 and subsequent generations from interspecific hybrids

A. Offspring from hybrids between tetraploid L. purpureum and diploid L. amplexicaule

1. *First origination of new L. intermedium*

a. The original plant, and its sister plants

After free flowering of *p*7 × *a*8 seven seeds were obtained, from which five plants developed. Two of these, 85_3 and 85_4, were in all respects

similar, and were completely normal, diploid *L. purpureum* plants, both with $2n=18$. Seed-setting and pollen fertility were perfectly good in both. Both plants were in complete agreement with the diploid line *p*2 corresponding to *p*7. There was thus nothing to indicate that the pure diploid *L. purpureum* line had not come out in these two plants after its passage through the tetraploid stage and the hybrids with *L. amplexicaule*.

The haploid *L. purpureum* gametes that rendered this possible were probably formed through regular meiosis of the *L. purpureum* chromosomes, at the same time as the unpaired *L. amplexicaule* chromosomes either all went to one of the poles or went with some of their number to one of the poles while the remainder were eliminated in the cytoplasm. The occurrence of two diploid *L. purpureum* plants in such a limited plant progeny indicates a way of restoring at will the chromosome number in autotetraploids to the original diploid number for special theoretical investigations, *e. g.*, to elucidate the way in which the diploid is affected by the genetic changes which have occurred in the autotetraploids after crossing and selection through a succession of generations with the aim of restoring fertility or other properties suffering from the tetraploid conditions.

The three other F_2 plants were all very dissimilar. One of them, 85_2, was apparently pure *L. purpureum*, though in appearance it resembled neither the diploids nor the tetraploids. In its appearance it seemed to be a slender plant of *p*7; but the shape, colour and size of the flowers, the shape and structure of the leaves, the appearance of the calyces, and the branching, which was evener and more abundant than is usually the case with *p*7, but not so long in relation to the stem as in *p*2 — all this was in a high degree intermediate, so the plant was suspected of being an autotriploid. The chromosome number was later found to be $2n=29$. Only two seeds were obtained. The pollen fertility was 46 %. Only one F_2 plant was obtained, though this was dwarfish (10 cm tall), sterile and not able to afford any clue to the nature of the mother plant.

Cytologically it is quite conceivable that the hypertriploid plant had been predominantly autotriploid. One of the gametes — the unreduced one and probably the ♀-gamete with the greatest resistance to the detrimental effect of two extra chromosomes — may conceivably have constituted, *e. g.*, a dyad cell, comprising only one of the nine univalents from I A. The other gamete would be haploid with a pure *L. purpureum* genome (*cf.* above). Thus, if in view of its appearance the plant is assumed to have had three *L. purpureum* genomes as well as two extra

chromosomes, probably from *L. amplexicaule*, it is nonetheless surprising that this plant could arise when it is impossible to produce autotriploid *L. purpureum* through direct crossing between tetraploids and diploids (BERNSTRÖM, 1953 a). If the plant actually was essentially autotriploid, the hybrid mother tissue would in the first place appear conceivably to be the factor that might make it possible for the plant to arise. A ratio of the chromosome numbers of 1 : 1,3 : 1 as between mother tissue, endosperm and embryo, which it is most natural to assume to have existed at the time when the triploid originated, coincides, moreover, more closely with the 1 : 1,5 : 1 normal for diploids and tetraploids than with 1,3 : 1,7 : 1, which is the result of the fertilization of a tetraploid plant with haploid pollen.

Plant 85_8 is reproduced in the middle of Fig. 30, with a plant of *p*7 to the left in the unfortunately rather unsuccessful picture. Fig. 32 shows that even the calyces of 85_8 had the appearance typical for *L. purpureum*. *p*25, used for comparison with the diploid appearance, has exactly the same shape of the calyces as *p*2, which was not available.

The 4th F_2 plant from $p7 \times a8$, 85_6, showed, surprisingly enough, distinct agreement, in respect of the majority of its features, with *L. hybridum*. The plant is reproduced to the right in Fig. 30. Few leaf indentations, late and slender development as well as other signs of weakness may conceivably have been connected with the hypotetraploid chromosome number of the plant, $2n=32$. The flowers resembled those from *L. purpureum* as closely as the flowers of *L. hybridum*, but the calyces, on the other hand, showed striking agreement with those of *L. hybridum*, only with the difference that those of 85_6 were somewhat smaller (*cf.* Fig. 32).

Unfortunately, the plant was almost sterile; only one seed was developed. The pollen fertility was only 7 %. The seed gave rise to a very stunted plant, which died at an early stage, and which from the taxonomical point of view was quite useless.

The mode of origin and the cytological nature of 85_6 will be discussed in the next chapter in connection with the corresponding questions concerning the last F_2 plant to be dealt with, 85_1. This was completely unlike 85_5, inasmuch as it agreed in a still higher degree with *L. intermedium* than 85_6 resembled *L. hybridum*. In its appearance 85_1 was very much like *L. intermedium*; thus if seen as an aberrant plant in a line of *L. intermedium* it would, without being suspected of being intermingled, have been regarded as a genuine member of the species, though certainly of a type not before seen (*cf.* BERNSTRÖM, 1953 b). The plant is re-

Fig. 30. To the left tetraploid *L. purpureum* (*p7*), in the centre plant No. 85_3, to the right plant No. 85_5 (see the text).

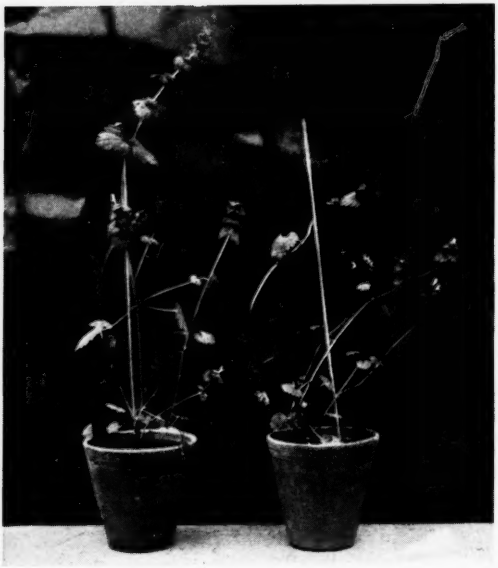


Fig. 31. To the left *L. intermedium* (*i22*), to the right plant No. 85_1 (see the text).

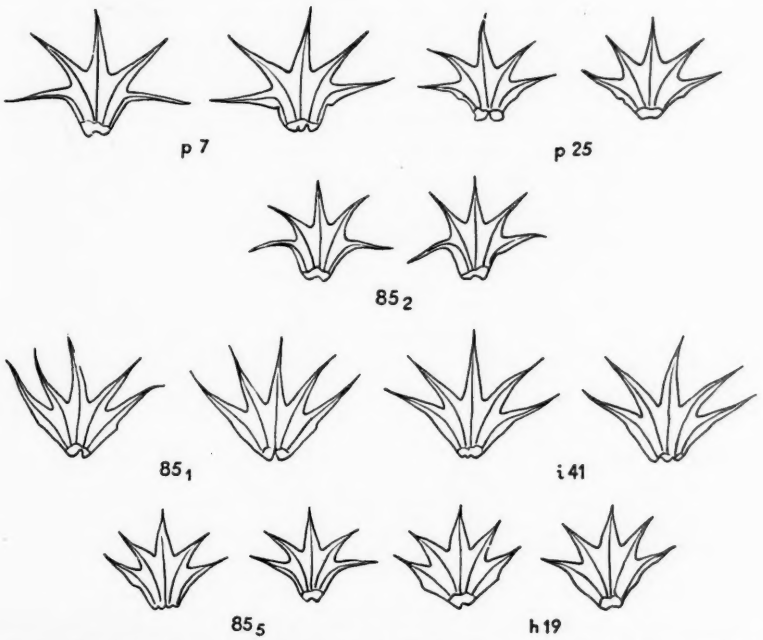


Fig. 32. Opened calyces from tetraploid *L. purpureum* (*p*7), diploid *L. purpureum* (*p*25), *L. intermedium* (*i*41), *L. hybridum* (*h*19), and three F_2 plants from tetraploid *L. purpureum*×diploid *L. amplexicaule* (85_1, 85_2, and 85_5). See the text. — ×1,8.

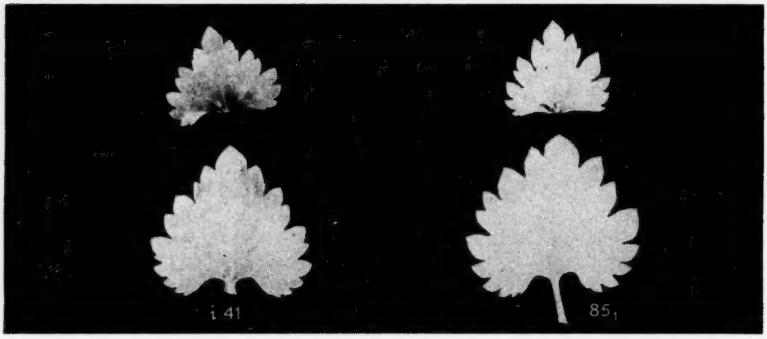
produced, unfortunately rather poorly, in Fig. 31, together with a normal specimen of *L. intermedium*.

85_1 was of more feeble growth than *L. intermedium*, which may have been a consequence of the fact that the plant was a hypertetraploid with $2n=39$. It grew to a height of 46 cm. The average height in two lines of *L. intermedium* was in the same year 49,9 cm and 47,1 cm, respectively. The leaves of 85_1 agreed well, as to shape, with those of *L. intermedium*, but the leaf indentations were coarser, more pointed and somewhat fewer in number than in the former. The flowers of 85_1 agreed almost completely with those of *L. intermedium*; they were only a trifle shorter, somewhat lighter on the lower part of the tube and, the most important point, they had a circle of hair in the tube, which *L. intermedium* lacks or manifests only as an indication. The calyces showed entire agreement with those of *L. intermedium*, being thus distinctly unlike the calyces of

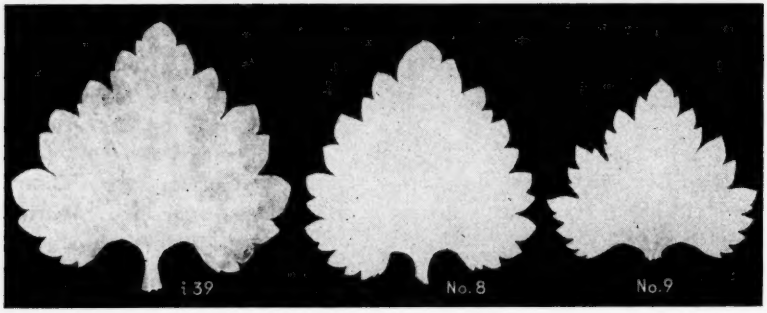
tetraploid *L. purpureum* and *L. hybridum*; *cf.* Fig. 32. The seed-setting for 85_1 was 7 % and the pollen fertility 65 %.

b. Subsequent generations of new *L. intermedium*

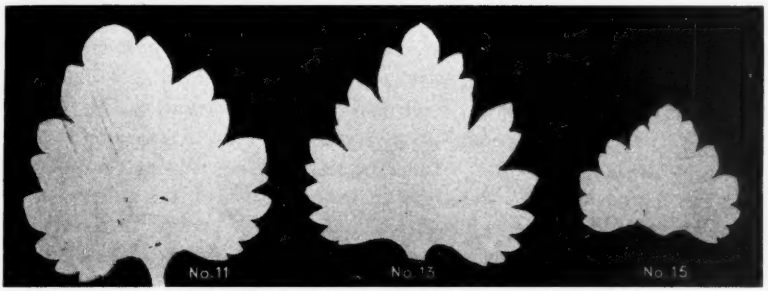
The progeny after 85_1 comprised 21 plants. The morphological variation between these was great, but in their appearance all the plants more or less strongly resembled *L. intermedium*. Two or three of them had appearances very closely coinciding with that of *L. intermedium*, some reminded one very strongly of this species in their appearance without showing any resemblances of detail, while some further specimens showed mostly in the shape of the calyces and the flowers and in various typical features in the leaves that they must be closely related to *L. intermedium*. In Fig. 33 is reproduced a sample of the different



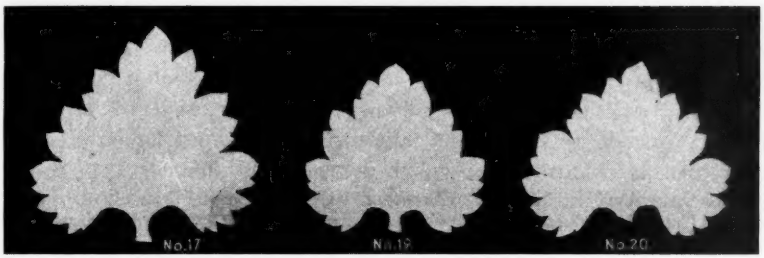
33 a



33 b 1



33 b 2



33 b 3

Fig. 33, *a* and *b*. — Fig. 33 *a*. Leaves from two different positions in the inflorescence of *L. intermedium* (*i*41), and of a *L. intermedium*-like plant (85_1) in F_3 from tetraploid *L. purpureum*×diploid *L. amplexicaule*. — Fig. 33 *b*. Leaves from *L. intermedium* (*i*39), and from eight plants in the offspring from plant No. 85_1. See the text.

types of leaf occurring, together with a leaf from the mother plant and one from *i*39 and *i*41, respectively, for comparison.

In the shape of the calyces all the plants agreed more with *L. intermedium* than with any other species, and the resemblance was in many specimens complete. Attention has already been drawn to the lesser sensitivity to genetic and cytological aberrations shown by the calyces than by the leaves, and the uniformity of the calyces in this progeny is *in et per se* a strong proof of relationship with *L. intermedium*. Fig. 34 shows calyces of the F_3 plants of which the leaves are reproduced in Fig. 33, with calyces from *i*39 for comparison.

Also the flowers of this F_3 generation showed a high degree of resemblance to those of pure *L. intermedium*. In two characters, however, all the plants differed distinctly from *L. intermedium*: all of them had

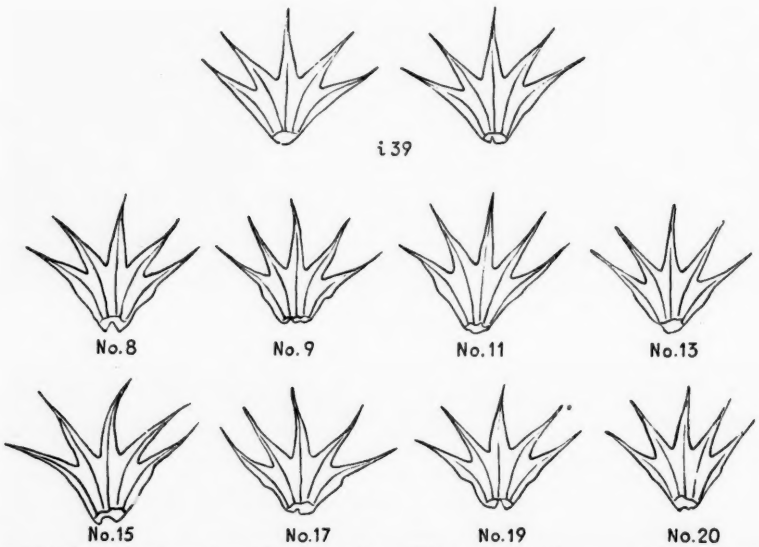


Fig. 34. Opened calyces from *L. intermedium* (*i*39), and from the same eight F_3 plants from tetraploid *L. purpureum*×diploid *L. amplexicaule* of which the leaves have been depicted in Fig. 33 *b*. — ×1,8.

a well-developed circle of hair in the tube of the flower, whereas *L. intermedium* is entirely or almost entirely without this; and further, the colour of the tube in the F_3 plants was not darker than that in other parts of the flower, which is, on the other hand, the case with *L. intermedium*. This shows that 85_1 cannot have been intermingled from *L. intermedium*.

In nine plants selected at random the chromosome number varied between $2n=36$ and $2n=38$; two individuals had 38 chromosomes, two had 37 and five had 36. There was obviously a tendency to revert to the euploid tetraploid number from the 39 chromosomes of the mother plant. The average for the seed-setting in the F_3 was in 19 plants 31,4 %, and for the pollen fertility 88,2 %. The fertility was thus considerably better than in the mother plant, which was probably in a high degree an effect of the more normal chromosome numbers. The three most fertile plants had a seed-setting of 58—66 %, and a pollen fertility of 95—99 %.

Summarizing, it may be said of this F_3 generation that it has convincingly shown that the resemblance between the plant 85_1 and *L. inter-*

medium was not an accident, but that genotypically the plant must have been closely related to *L. intermedium*, though not identical with the latter. The mother plant with progeny has therefore been designated *ni*1 (=new *intermedium* 1), in order to distinguish it from the other origination of *L. intermedium* (*ni*2; *cf.* below).

The above observation is important for an assessment of the question concerning the origin and real nature of the two in appearance quite different *L. hybridum*-like (85_5) and *L. intermedium*-like (85_1) F_2 plants. As the F_1 hybrids had flowered freely, one had to reckon with the possibility of cross-fertilization. The two diploid *L. purpureum* plants and also the triploid *L. purpureum*-like plant were in all probability products of self-fertilization, for there were no specimens of either diploid or tetraploid *L. purpureum* in the greenhouse. In the autumn, during the time the F_1 plants were flowering, the greenhouse was visited regularly by a single, rather small bumble-bee, that found its way to the flowers of all species and species hybrids to be found there, *i. e.*, besides tetraploid *L. purpureum*×diploid *L. amplexicaule*, chiefly *L. intermedium*, *L. hybridum*, *L. hybridum*×tetraploid *L. purpureum* and the F_2 of the latter. The flowering of *L. amplexicaule* was exclusively cleistogamic.

The keeping in the F_2 from 85_1 of the characters typical for *L. intermedium* must, however, imply genetically that if the F_2 plant in question originated after cross-fertilization with *L. intermedium*, then it has been possible for a gamete genotypically and chromosomally fairly equivalent to the *L. intermedium* gamete to develop and function in the F_1. If, on the other hand, 85_1 has appeared after self-fertilization, then either two genotypically and chromosomally roughly equivalent gametes may conceivably have functioned, or else the two gametes may have been different while at the same time being complementary to each other, so that the product nevertheless turned out to be a zygote with about the same genotypical composition as that characterizing *L. intermedium*.

If the F_2 plant 85_1 originated after cross-fertilization, a hyperdiploid female gamete (18+3) must have existed in the triploid F_1 plant, for the fertilizing *L. intermedium* gamete must in reason be assumed to have been normal. Without having devoted a more detailed study to the later stages of meiosis and the behaviour of the univalents in the F_1 it is rather hazardous to try to explain how such an ovum might have been formed. Dividing univalents in I A have never been definitely observed in *Lamium*, and it is therefore rather unlikely that non-separation of split univalents in either of the divisions should be the cause of the extra

chromosomes. None of the pairing conditions found in I M can give more than 19 chromosomes if the separation of the bivalents is conceived as normal at the same time as all the univalents go to the same pole. It is conceivable that the ovum was formed after partial asynapsis or in some other equally irregular way; the seed appeared in October or November, possibly on a plant that had been cut for bud-fixation, and asynapsis was observed in *L. intermedium* the same autumn in a similarly truncated plant; *cf.* BERNSTRÖM, 1953 b.

Assumptions of the same nature as those stated above are, however, required also for an explanation of the appearance of the gametes that must be conceived for a possible self-fertilization. One may also make the assumption that the F_1 plant was aneuploid, with 29 chromosomes. In a 29-chromosome hybrid plant it must occur, though rarely, that gametes with 19—20 chromosomes are formed through a concentration of all the *L. amplexicaule* univalents at the one pole together with the one or both of the supernumerary chromosomes of *L. purpureum* it would then be a matter of. If 85_1, on the other hand, arose after cross-fertilization, than its mother plant must by the same reasoning have had $2n=30$. Five out of the 14 hybrid seeds giving rise to the F_1 did actually derive from 37-chromosome *L. purpureum* plants, and the remaining nine seeds from 36-chromosome plants; ova with in any case two supernumerary chromosomes were probably occasionally formed in the 37-chromosome plants.

From what has been adduced above it would seem impossible to decide cytologically whether the plant 85_1 arose through self-fertilization or through cross-fertilization. But however this may be, its appearance is a proof of the nature of *L. intermedium* as an allotetraploid between *L. purpureum* and *L. amplexicaule.*

This being the case, it is for several reasons highly improbable that the *L. hybridum*-like F_2 plant (85_5) arose after self-fertilization. *L. hybridum* and *L. intermedium* are so different from each other, *e. g.*, in the appearance of the calyces, that it is inconceivable that a plant in a high degree resembling *L. hybridum*, and moreover tetraploid, should segregate from a hybrid from which — as it has proved — one should rather expect *L. intermedium*-like or *L. purpureum*-like progeny or intermediate types. Three genomes from *L. purpureum* and one from *L. amplexicaule* enter in the hybrids between *L. intermedium* and tetraploid *L. purpureum*, which do not at all resemble *L. hybridum.*

Eight plant-progenies originating from plant No. 85_1 were raised in the F_4, and two of these were followed even as far as to the F_7. The

most seed-fertile plant in each line was then selected in the first place in each generation. The best line in this respect had, already in the F_4, an average seed-setting of 59,0 % (n=13) as compared with 59,7 % and 48,0 %, respectively, in two pure lines of *L. intermedium*. This relatively high fertility was then on the whole maintained in later generations; thus the best of the two lines in the F_7 had 71,1 % seed-setting (n=10) as compared with 79,2 % for *i*39 (n=9). Also in a couple of other lines the fertility increased successively, while in others, again, it remained about half that occurring in *L. intermedium*. Between the plants in almost all the lines, however, the seed-setting varied far more than is the case in *L. intermedium*, indicating an inability to achieve rapidly any cytological or genetic stability.

As regards the appearance of the plants, too, the variation was very evident, both as between lines and as between plants within the lines. Each line was propagated by only one mother plant in each generation, and not until the F_6 and the F_7 did the correlation between the appearance of the mother plants and their respective progenies become more clearly discernible, even if in these generations, too, the variations between the plants were rather considerable. It would take us to undue lengths to give a more detailed documentation of this, as indeed, to go at all into detail concerning these later hybrid progenies; it must suffice to give as a general conclusion the opinion that a variation in fertility and morphology as great as that found here, and one so lasting, may be suspected of being due to a hybrid origin of the plant 85_1 rather than to an origin through self-fertilization.

This opinion is also supported by the circumstance that even as early as in the F_3, and thereafter in all later generations, there occurred plants which in a high degree agreed in appearance with *L. intermedium*. On account of the morphological variability between pure lines of *L. purpureum* and in a still higher degree of *L. amplexicaule* (*cf.* BERNSTRÖM, 1952, 1953 b), one must expect that allotetraploids between these two species will each have their own individual appearance, according to the appearance of the respective parent lines. The following chapter gives an instance of this; it is also confirmed by the different appearances in the triploid hybrid combinations. It would thus be a remarkable coincidence if a new appearance of *L. intermedium* within the experimental material should include lines of the parent species, which must in a high degree have resembled those which once produced the original *L. intermedium*. Fig. 35 shows as typical an exponent as possible for each of the two lines cultivated as far as the F_7 (Nos. 125 and

126); apart from the fact that it is shorter, the plant designated 125 shows a high degree of resemblance to that immediately to the left. which represents *L. intermedium*, while the plant denoted 126, which represents the line with the better fertility in the F_7 (*cf.* above), is characterized by a rather deviating appearance.

Finally, it should be pointed out that the marked variation in fertility and morphology characterizing the progenies of the plant 85_1 constitutes a striking contrast to the uniformity characterizing the progeny of the second allotetraploid between *L. purpureum* and *L. amplexicaule*, *ni*2 (*cf.* below). Such a difference would be understandable if *ni*1 comprised two chromosome-structurally different genomes of *L. amplexicaule* as well as genotypically different genomes of the two parent species, while *ni*2 was composed of only one line from each species. Probability thus favours the assumption that *ni*1 arose through cross pollination with *L. intermedium*.

2. *Second origination of new L. intermedium*

In C_1 of *L. purpureo-amplexicaule* 2 one plant differed from the others in its greater height and considerably better seed fertility, this being 49 % as against an average of 1,9 % for the others (Table 16). The pollen fertility was 86 %. The plant was found to have 37 chromosomes. The appearance of the plant did, certainly, show quite clearly that it belonged to the line, but on the other hand there was some divergence in the leaves, and even more in the calyces, from the appearance typical for *L. purpureo-amplexicaule* 2, approaching instead that characteristic of *L. intermedium*.

The progeny of this plant was called *new intermedium* 2 (abbreviated to *ni*2). Fourteen plants had $2n=36$, while one had $2n=37$. The average height was 45,2 cm, as compared with 33,9 for the hexaploid sister plants. Fourteen plants of *i*39 had an average height of 70,2 cm. The appearance of the tetraploids showed entire agreement with that of the mother plant; and it was more reminiscent of that of *L. intermedium* than of any other species. A feature diverging from the latter was the lesser height, which also in subsequent generations remained about $^2/_3$ of that for *i*39. The branches, moreover, approximated in length more closely to the stem than is the case with *i*39. The leaves were smaller than in genuine *L. intermedium*, with the base rather square than cordate, larger leaf indentations, and lighter colour. The flowers, too, were somewhat smaller, and the points of the sepals rather more

Fig. 35. From left to right: New *L. intermedium* (=*ni* 2); natural *L. intermedium* (=*i*39); representatives of two lines (in F_7) of new *L. intermedium* (=*ni* 1); see the text.

Fig. 36. From right to left: diploid *L. amplexicaule* (*a*13), tetraploid *L. purpureum* (*p*42), new *L. intermedium* (*ni*2), and *L. purpureo-amplexicaule* 2. The two latter ones constitute the allotetraploid and the allohexaploid, respectively, of the two former.

directed outward. *ni*2 withers about one week before *L. intermedium*. Fig. 36 shows a plant of *ni*2 (denoted 112 in the picture) as well as a typical plant of *L. purpureo-amplexicaule 2* (denoted 95) and a plant of each of the parent lines (42 for *p*42, and 13 for *a*13). In the figure one sees what also immediately struck one when examining the living material, *viz.*, that *ni*2 was bigger than either of the parent lines. Fig. 35 shows from left to right a plant of *ni*2, *i*39, and specimens from two lines of *ni*1.

The average seed-setting in three successive generations after the tetraploid plant was 36,7, 39,9 and 49,4, which constituted 46 %, 56 % and 62 %, respectively, of the seed-setting of the standard *i*39. The corresponding percentages for the pollen fertility of *ni*2 were 86, 97 and 92. As each generation derived from the most seed-fertile plant of that immediately preceding it, it is conceivable that the increase in fertility was not due to chance, but reflected a successively improved genic and/or cytological balance in the line. There still occurred, even in the third tetraploid generation, a slight but distinct morphological variation between the plants, inasmuch as some diverged in a way reminding one of the more slightly aberrant plants in *L. intermedium* (BERNSTRÖM, 1953 b). The uniformity between the plants was otherwise good, and much better than was the case even in the later generations of *ni*1.

The considerably inferior seed-setting in *ni*2 as compared to that in *i*39 may conceivably be due to two different circumstances. The original fertility of genuine *L. intermedium* may thus have improved through mutations combined with occasional interlinear hybridizations; structural recombinations in consequence of heterogenetic pairing may possibly also have been of importance. But it is highly probable that the genetic constitution of the parent lines may be of importance for the degree of fertility of the allotetraploid. Tetraploid intraspecific hybrids in many species, including *L. amplexicaule* and *L. purpureum*, have been characterized by considerable differences as regards improvement in seed fertility over that of the parent lines (*cf.* BERNSTRÖM, 1954); and in all probability also interspecific hybrids, *i. e.*, allotetraploids, may behave in a corresponding way, as has proved, moreover, to be the case in *Triticale* (MÜNTZING, 1939).

Unfortunately, only scanty data are available concerning the seed-setting in the parent lines of *ni*2 in plant generations contemporary with the study of *ni*2. From a previous paper it will be seen, however, that the average value for the seed-setting of *a*28 in three generations

was 51,5 %, and that of *p*42 in six generations 29,8 % (BERNSTRÖM, 1954, Tables 1 and 8). With a corresponding average value of 42,0 % in three generations, *ni*2 must be assumed to occupy an approximately intermediate position between its parents.

The only investigation of the meiosis in *ni*2 it was possible to make was made on a plant with good seed fertility but with low pollen fertility from the second tetraploid generation. Sixteen PMC's had in I M 18 bivalents, while five had 17 II+2 I and one had 16 II+4 I. Thus, at least predominantly, the chromosomes pair in bivalents, as is the case with *L. intermedium*.

In order to test the cytogenetic relationship between *ni*2 and *L. intermedium*, F_1 hybrids between them were produced; the crossability was good. The seed-setting in *ni*2, F_1 and *i*39 was 39,9, 50,0 and 71,8 %, respectively. The pollen fertility was 95,9, 91,4 and 98,7 %, respectively. One of the 11 F_1 plants was practically sterile, weak and morphologically markedly aberrant (2 % seed-setting and 25 % pollen fertility); if we omit this the respective values for the F_1 hybrids become 54,8 and 98,1. The remaining hybrids were well-developed and agreed more with *i*39 than with *ni*2, both in point of appearance and in respect of height of plant.

The fertility in the hybrids thus confirms the conclusion that *ni*2 and *i*39 must really be accounted as belonging to the same species, and that a close chromosome-structural homology between the lines must be assumed to exist. Attempts to study the chromosome pairing in the hybrids unfortunately proved unsuccessful.

As regards the question of the mode of origin of *ni*2, it may be remarked that owing to lack of space the material of *p*42×*a*13 treated with colchicine was placed in a separate greenhouse situated about 150 meters from the house with the other *Lamium* material, and without wild-growing *Lamium* plants in the vicinity. Cross pollination was thus as regards the hybrids reasonably out of the question. An assumption of cross pollination is also contradicted in the first place by the fact that the appearance of the tetraploid original plant showed close agreement with the *in et per se* characteristic features of the hexaploid sister plants, which in many respects bore the stamp of the peculiar appearance of *p*42, and in the second place by the circumstance that the morphological variation within *ni*2 was rather slight and remained well within the frame of the original appearance of the line. The difference in this respect as compared with *ni*1 was striking.

From all this one is obliged to draw the conclusion that *ni*2 originated

in a triploid flower, independently of the colchicine treatment, through the functioning of two hereditarily approximately equivalent diploid gametes. These must have originated through an at least on the whole regular distribution of the chromosomes of *L. purpureum* in I A, together with accumulation of all the *L. amplexicaule* chromosomes at the one pole. The variation between the plants, lasting through several generations, may conceivably have had its origin in heterogenetic pairing in the one or both of the pollen mother-cells, with thereby conditioned genetic and cytological irregularities, to which the rather high frequency of univalents in the only cytologically investigated plant may have testified.

Summarizing, it may be said that the observations made in connection with *ni*2 confirm the conclusions from *ni*1 concerning the nature of *L. intermedium*. They also demonstrate the possibility that this species originated from a triploid hybrid between the two parent species; which of the species was doubly represented in the latter may be a matter of indifference, but the prospects of such a hybrid arising in natural conditions are probably extremely slight, as no natural autotetraploid of either species is known, and unreduced gametes have never been observed in the pure species, and are, moreover, probably in themselves very rare.

B. Investigations on F_2 generations from L. hybridum×tetraploid L. purpureum

1. *F_2 from hybrids of normal growth*

a. Morphology and fertility

The species hybrids in *Lamium* have in general been very markedly sterile, and it has consequently been difficult to study the F_2 generations. Such studies have, moreover, not as a rule been of primary interest; thus only few F_2 generations have been studied. In connection with an investigation of the seed-setting in different line combinations of *L. hybridum*×tetraploid *L. purpureum* (*cf.* p. 26), however, an opportunity was afforded to collect a sufficient quantity of seed for a fairly representative investigation of the properties of the F_2 after the vigorous type of the F_1 hybrids. Thus, the F_2 hybrids from the following combinations were studied together: 31 plants from $h21 \times p42$, 27 plants from $h36 \times p42$, 76 plants from $h21 \times p30$, and 40 plants from $h36 \times p30$.

As was expected, the morphological variation in this material was very great, so that not two plants were alike; the majority of the plants, however, were on the whole reminiscent of the F_1 hybrids, with greater or less stress, in respect of appearance as well as in the shape of the different organs, upon the one or the other of the species entering. Some few plants reminded one very strongly of one or the other of the parent species, but none agreed completely with either of these. Although difficult to judge, a rather greater number appeared to resemble *L. purpureum* in the same degree as others agreed with *L. hybridum*. This may be due to a stronger elimination during meiosis in the F_1 of chromosomes from the genome which is inhomologous with that of *L. purpureum*, than of chromosomes deriving from this species (*cf.* p. 12). There was not a single plant that agreed more with any other species than with *L. hybridum* or *L. purpureum*.

The F_2 plants were investigated in respect of their seed-setting, pollen fertility, height of plant, vigour and time at which flowering commenced. As it was not possible to investigate the seed-setting more accurately than usual — thus only on the flowers in the two nethermost whorls on the stem —, plants with poor seed-setting have been designated as seed-sterile even if their seed-setting was better than, *e. g.*, that of the F_1 hybrids. In order to get a better criterion of the vigour of the plants than that constituted by the height alone, and since for divers reasons it was not appropriate to weigh the plants, each plant was evaluated after ocular scrutiny. A scale of 10 degrees was used, so that the weakest plants were designated 1 and the best 10; in connection with the evaluation the F_1 plants and the parent lines were used for comparison.

In Table 17 the averages of the different properties for each hybrid progeny are calculated. As regards the seed-setting, there proved to be, proportionately, considerable differences between the progenies, but the seed-setting was in all of them far better than in the F_1 hybrids (*cf.* p. 26); as regards $h36 \times p30$, in which the number of flowers per plant in the F_1 hybrids is known, it may thus be calculated to have been about 25 times greater. The high degree of sterility in the F_1 hybrids has been earlier shown to be preponderately of a chromosomal nature (p. 27); the marked improvement of the seed-setting in the F_2 must thus be assumed to be caused by an important general improvement in the chromosomal balance of the gametes, which in its turn must have arisen through a corresponding improvement in the F_2 plants, resulting in a more regular meiosis than in the F_1.

TABLE 17. *Average values of some characteristics in F_2 of L. hybridum ×tetraploid L. purpureum.*

Cross combination	Seed-setting		Pollen fertility		Height of stem		Vigour		Florescence time *	
	$\bar{x} \pm m$	n	$\bar{x} \pm m$	n	$\bar{x} \pm m$	n	$\bar{x} \pm m$	n	$\bar{x} \pm m$	n
h21 × *p42*	0,81 ± 0,259	31	30,0 ± 2,73	31	42,1 ± 1,49	30	8,71 ± 0,181	31	9,38 ± 0,570	24
h36 × *p42*	1,90 ± 0,537	26	36,3 ± 4,75	27	40,8 ± 1,47	26	7,78 ± 0,279	27	12,35 ± 1,140	23
h21 × *p30*	2,53 ± 0,631	75	46,7 ± 2,32	76	39,8 ± 1,13	76	7,72 ± 0,193	76	8,99 ± 0,442	67
h36 × *p30*	4,98 ± 1,591	39	44,8 ± 3,98	40	34,7 ± 1,27	40	7,75 ± 0,234	40	11,00 ± 0,538	33

* Some plants were pricked out after the majority, and were thus not included in the table.

The two combinations with *p*30 had a better seed-setting than the two others, as was also the case with the F_1 hybrids (*cf.* p. 26). In a partition of the degrees of freedom *a priori* the combinations with *p*30 were contrasted with those with *p*42 in an analysis of variance; the quotient between the mean squares for »between» and »within» was 4,34 ($0{,}05 > P > 0{,}01$). Thus with rather great probability the F_2 generations from *p*30 are more seed fertile than those from *p*42.

It emerges further from Table 17 that the pollen fertility in the two combinations with *p*30 is greater than in the two others. An analysis of variance analogous to the above-mentioned one results in a quotient of 14,46 ($P < 0{,}001$) between the mean square for the combinations with *p*30 as against those with *p*42 and that for »within». The pollen fertility in the F_2 generations of *L. hybridum*×*p*30 was thus significantly superior to that of *L. hybridum*×*p*42. Also in the F_1 the pollen fertility in *h*21×*p*30 was better than in the combinations with *p*42, while *h*36×*p*30 was here only at the same level as these.

Thus *p*30 obviously had a better effect upon the fertility of the hybrids with *L. hybridum* than *p*42; the chance that it should be one and the same line that in both the F_1 and the F_2 gave a better seed fertility was 1 in 36; and the probability that the same line should give both better seed-setting and better pollen fertility in the two generations was only 1 in 1296. The pollen fertility in the F_1 was, certainly, not so much better in the combinations with *p*30 than in those with *p*42 that *p*30 with the above-mentioned significance might be said to excel *p*42 as a fertility-promoting line, but the evidence for its superiority was nevertheless strong.

It is interesting to find that the stimulating effect of *p*30 applied to both the pollen fertility and the seed-setting. From previously published data (BERNSTRÖM, 1954, Tables 7 and 8) it may be seen that the pollen fertility in *p*42 rather tended to be better than that for *p*30 than *vice versa*, but that the seed fertility in *p*30 was distinctly the better as between the two lines. The — especially during 1945—2 — very good seed-setting in *p*30 has been assumed to have been caused by spontaneous cross pollination with other lines (*cf.* the paper mentioned), and heterozygous material of *p*30 has at least in part entered also in the hybrids with *L. hybridum*. Whether the favourable effect of p*30* upon the fertility of the species hybrids was to be referred to increased fertility in this line itself, caused by heterozygosis, we may leave an open question, although it appears quite conceivable; what in the above-adduced results is of the greatest interest, however, is that genic differ-

ences between the lines of *L. purpureum* may be clearly reflected in the fertility of the species hybrids, which must nevertheless in a high degree suffer from the irregular meiosis and the thereby conditioned markedly irregular chromosome distribution among the gametes. It is not possible at present, however, to infer whether the influence on fertility be haplontic or diplontic.

Also as regards other properties listed in Table 17 there occur more or less significant differences between the different progenies, caused obviously by differences between the parent lines. The superiority of $h21 \times p42$ in plant vigour was unexpected, however; from a general point of view one would not have expected superiority in a single combination in a quality like vigour. The explanation may be assumed to lie more in undue consideration being given to the actual heights of the plants, and perhaps also to the finer mode of growth by which, each in its way, both *h*21 and *p*42 are characterized, than in a real superiority in respect of vigour in this line combination.

b. Chromosome-number variation

The continuous variation in chromosome number extended between $2n=33$ and $2n=44$, besides which one plant each of $2n=29$ and $2n=54$ occurred (*cf.* Table 18). Between the average values for the chromosome number of each hybrid combination there were only slight and quite insignificant differences; the material has thus accordingly been given as one sample in the table. The mean chromosome number for this is 36,8, which is somewhat higher than the corresponding figure for the spontaneous occurrence of aneuploids in autotetraploid *L. purpureum* and also tetraploid *L. amplexicaule* (*cf.* BERNSTRÖM, 1954, Tables 4 *a* and 12 *a*). If, however, one considers the distribution of the aneuploids around the euploid number, one finds that only 61,6 % of the number of aneuploids in the hybrid progenies have more than 36 chromosomes, while the corresponding figure for tetraploid *L. purpureum* is 72,1 % and for the entire *L. amplexicaule* material 86,1 % (or 92,3 % for the line with the highest aneuploid proportion, and the highest mean chromosome number). The nonetheless higher mean chromosome numbers in the hybrid progenies are due to the fact that the shift towards hypertetraploidy is much more extended than towards hypotetraploidy; if only the continuous variation is taken into consideration, then the classes with supernumerary chromosome numbers are eight, while those with deficient chromosome numbers are three, despite the fact that the class with 35 chromosomes

TABLE 18. *Chromosome number variation in the F_2 generations from L. hybridum×tetraploid L. purpureum.*

Cross combination	Somatic chromosome numbers														n	x̄
	29	33	34	35	36	37	38	39	40	41	42	43	44	54		
*h*21×*p*42			1	7	7	1	5	4	3	1	—	—	1		30	37,3
*h*36×*p*42			2	9	4	3	5	2	—	1	—	—	—	1	27	37,1
*h*21×*p*30	1	3	5	15	13	20	9	6	1	1	—	2			76	36,5
*h*36×*p*30		2	3	5	10	7	3	4	2	1	2				39	36,9
Total	1	5	11	36	34	31	22	16	6	4	2	2	1	1	172	36,8

is that most represented. — The variation in chromosome number in the hybrid progenies is thus, certainly, very considerably greater than the corresponding variation in the autotetraploids, but it does nevertheless give the impression of being on the whole regulated, proportionately, in a way corresponding to that obtaining for the latter.

As has been shown earlier, the F_1 hybrids have in general three structurally homologous genomes and one odd genome, which in I M, as a rule, entailed the occurrence of 9—14 univalents and 9 trivalents or bivalents. One may thus in I A expect an extreme distribution of 9—18 *L. purpureum* chromosomes (or chromosomes homologous with these) and 0—9 odd chromosomes; we abstract here from incidental pairing between chromosomes of the odd genome and those of the others. The chromosome number in the gametes may thus be expected to vary between the extremes $n=9$ and $n=27$; the mean chromosome number will, however, in all likelihood be somewhat below $n=18$ in consequence of the very probable elimination of univalents, as the case has proved to be, *e. g.*, with triploid *Allium*, *Hyacinthus* and *Crepis* (LEVAN, 1936; CHUKSANOVA, 1939, from DARLINGTON and MATHER, 1949).

Since the variation in chromosome number in the F_2 ranges between 29 and 54, and does not begin with $2n=18$ or the figures nearest this, this may in the first place be due to a marked elimination of the more aberrant gametes, where genic and cytological unbalance acts all the more strongly the lower the chromosome number is, and in the second place to the fact that gametes with about 18 chromosomes are by far the commonest. The class with the greatest number of individuals is $2n=35$, despite the fact that $2n=33$ is the lowest number in the continuous variation; these circumstances bear out the above assumption that gametes with <18 chromosomes are the commonest, and show at

the same time that the factors preventing the extension of the hypotetraploid chromosomal variation are obviously very strong. In contradistinction to the variation in the hypotetraploid direction, the continuous variation towards increasing chromosome numbers would appear to be easily extended some further steps with an increased plant material. The single plant with 54 chromosomes in all probability originated from an unreduced and a reduced gamete.

From other plant material one knows the importance for the vitality of the gametes of their chromosome number and the fatal effects of even lesser deficiencies and duplications; the difference in sensitivity to disturbances of this kind as between male and female gametes is also a well-known fact. How dependent upon the chromosomal equipment and the genotype of the gametes is the growing capacity of the pollen tubes is also well known from, *inter alia*, *Datura* (*e. g.*, BUCHHOLZ and BLAKESLEE, 1930) and *Triticum* (O'MARA, 1950). Factors like those mentioned must be of great importance for the distribution of the chromosome numbers in the F_2, especially in the hypotetraploid restriction of the latter. As will be shown in more detail later, the plant vigour is not improved with increasing chromosome number, rather does it seem on an average to be somewhat better in plants with lower chromosome numbers and reduced with increasing chromosome number. This might imply that the lower the chromosome number in especially the male gametes, the stronger will be the elimination of the hereditary defective ones, and the better will be the resulting zygotes. The heights of the plants, taken as a by all means rather unsatisfactory criterion of their vigour, are, moreover, greatest in the classes with the lowest chromosome numbers and decrease significantly in the measure in which the latter increase. Observations like these do not indicate that zygotic lethality is an important factor in restricting the hypotetraploid chromosome number variation.

The extensive variation in the chromosome numbers of the F_2 progenies must presumably be favoured by a high degree of freedom from competition between the male gametes due to a low proportion of functioning ones in combination with small amounts of pollen on the stigmas owing to defective and often failing self-fertilization; this latter defectiveness has frequently been observed. An important contributory cause of, *inter alia*, the fact that the group with $2n=35$ may show the greatest number of individuals lies presumably in these circumstances. In the progenies of hypotetraploid plants of autotetraploid *L. amplexicaule* and *L. purpureum* the group with $2n=36$ was always by far the

biggest, and included, moreover, quite as many hypertetraploid as hypotetraploid plants (BERNSTRÖM, 1954). Since gametes with < 18 chromosomes must be formed in the majority in hypotetraploid plants, the proportion being increased by incidental elimination of univalents, the above-mentioned distribution of the chromosome numbers in the autotetraploids must be based upon a marked reduction of hypodiploid gametes due to competition. There is no reason to suspect that the greater genic variation among the gametes of the species hybrids brought about by the presence of at least two kinds of genomes instead of one, as in the autotetraploids, has contributed to the appearance of hypodiploid gametes better able to survive competition than those of the autotetraploids; rather may one presume the contrary to be the case.

c. Effects of the numerical chromosome variation

In the present material a rather detailed study has been devoted to the dependence of the seed-setting, the pollen fertility, the height and the vigour of the plants upon the chromosome number. On account of the differences between the progenies shown in Table 17, the correlations have been calculated separately in each progeny; *cf.* Table 19. In two of the progenies the seed-setting shows a clearly positive correlation with increase of the chromosome number, but in the two other progenies it is indifferent to the latter; the pollen fertility, on the other hand, improves significantly with an increase in the chromosome numbers. As regards the height of the plants, a diminution with increasing chromosome number is evident, except for one of the progenies where the

TABLE 19. *Coefficients of correlations between chromosome number and different characteristics in the F_2 from L. hybridum×tetraploid L. purpureum* (*cf.* the text).

Cross combination	Seed-setting r	n	Pollen fertility r	n	Height of stem r	n	Vigour r	n
*h*21 × *p*42	+0,294	30	+0,575 ***	30	−0,166	29	−0,010	30
*h*36 × *p*42	−0,003	26	+0,324 (*)	27	+0,122	26	−0,209	27
*h*21 × *p*30	+0,214 (*)	74	+0,414 ***	75	−0,233 *	75	−0,138	75
*h*36 × *p*30	+0,012	38	+0,377 *	39	−0,270 (*)	39	+0,005	39

Levels of t-significance of correlation coefficients denoted thus:
(*) $P < 0{,}1$. — * $P < 0{,}05$. — ** $P < 0{,}01$. — *** $P < 0{,}001$.

correlation is positive, and the same negative tendency is observable in respect of the vigour of the plants.

As a complement to the analysis of the two last-mentioned properties the relative values, *i.e.*, the absolute value as a percentage of the mean value for the cross combination in question, has been calculated for each plant, and the whole material has been grouped according to the chromosome number. Although the method is approximative, it may serve to illustrate the trend in the material. Results:

$2n=$	29—35	36—37	38—39	40—54
		Height of plant		
	$107{,}04 \pm 2{,}65$	$99{,}70 \pm 2{,}81$	$96{,}30 \pm 4{,}05$	$93{,}00 \pm 4{,}88$
	n=52	n=63	n=38	n=16
		Vigour		
	$102{,}83 \pm 2{,}18$	$100{,}95 \pm 2{,}23$	$96{,}95 \pm 4{,}14$	$96{,}06 \pm 4{,}18$
	n=53	n=64	n=38	n=16

The difference between the extreme groups is rather significant in respect of the height of the plants, but less in respect of their vigour. The continuous reduction of the latter property with increasing chromosome number indicates, however, the existence of a negative correlation here, too.

As regards the commencement of flowering, it was not possible to observe any clear tendency to a change in connection with increasing chromosome numbers.

To complete the investigation of the correlations between seed-setting, pollen fertility and chromosome number, the two first-mentioned properties were correlated within each progeny.

The correlation coefficients were:

$h21 \times p42$	$h36 \times p42$	$h21 \times p30$	$h36 \times p30$
$+0{,}642^{***}$	$+0{,}551^{**}$	$+0{,}564^{***}$	$+0{,}528^{***}$

Thus, as was expected, the correlation between the two criteria for the fertility was good, and it would probably have been still better if the self-fertilizing capacity of the flowers had not often been considerably poorer than is the case in the parent species. Impaired self-fertilization must of course entail reduced seed-setting, and as the variation in respect of self-fertilizing capacity as between the plants was very distinct, the seed-setting, of which it was possible to make only a rough estimate (*cf.* above, p. 79), must be presumed to reflect the real seed-setting capacity of the plants in very different degrees.

Since the tendency to increased seed-setting with increased chromosome number is nevertheless clear (Table 19), at the same time as a significant positive correlation exists on the one hand between the chromosome number and the pollen fertility and on the other hand the pollen fertility and the seed-setting, this shows that there is a positive correlation between an increase in the chromosome number and an improvement of the fertility as a whole.

Thus in this F_2 material the latter is regulated both by genic factors, noticeable in the superiority of the combinations with *p*30 in the F_1 as well as in the F_2, and by chromosomal factors, manifested in the F_2.

As regards the improvement in fertility, especially the pollen fertility, with increasing chromosome number, it is well known from investigations especially on *Datura*, but also *Lycopersicum*, *Zea*, *Matthiola*, *etc.* (summary by SANSOME and PHILP, 1939), that the loss of a chromosome has a more drastic effect than has the addition of an extra chromosome upon the vitality of the zygotes as well as upon the viability of the gametes and especially of the pollen. This applies far more to diploids than to tetraploids, as has been demonstrated in detail on, *inter alia*, *Dactylis* (MÜNTZING, 1937). In the latter, as also in autotetraploid *Lamium purpureum* and *L. amplexicaule*, the fertility was, as measured in both its manifestations, greatest in the euploid plants, and diminished, as a rule, more with decreasing than with increasing chromosome number (BERNSTRÖM, 1954).

The lower the chromosome number in the F_2 plants, the more gametes, proportionately, must be formed with a deficit of one or more chromosomes. With increasing somatic chromosome number the proportion of the thus deficient gametes is reduced, and that with surplus or approximate balance increases. The proportion of deficient gametes may be expected to be in the majority even in rather pronouncedly hypertetraploid plants, also in consequence of the elimination of univalents. In this way the proportion of viable gametes may, however, be expected to rise with increasing chromosome number; a similar explanation has been developed by MÜNTZING (1937) for a corresponding improvement in fertility in *Dactylis*, although on the assumption that it was here a matter of an autotetraploid.

As the seed-setting shows a tendency to increase with the chromosome number, this may imply that also the viability of the female gametes, although more insensitive to cytogenetic disturbances than the pollen, would be somewhat improved by an increase in the chromosome number. The tendency mentioned is, however, rather slight, and cytogenetic

irregularities affect so many vital functions in the life-processes of plants which also in varying degrees exercise a direct or indirect influence upon the result of the seed-setting, that a correlation between the latter and the chromosome number may be conceived partly to be only indirectly caused. Interplay between haplontic and diplontic sterility is here also likely; it is known that diplontic sterility may be caused by chromosomal unbalance (*cf.* MÜNTZING, 1937; LAMM, 1945; EINSET, 1947).

The explanation of the on the whole negative correlation between chromosome number and the vigour and height of plant, respectively, that was advanced in the discussion of the chromosomal variation (p. 84) does not contradict the explanation of the improvement in fertility with increasing chromosome number. For even if the plants with lower chromosome number are characterized by a better genic balance, their gametes nevertheless get on an average a lower chromosome number than do gametes from plants with higher number, and the possibility of deficiencies, also including whole chromosomes, must consequently be greater for the first-mentioned category.

Among the morphological investigations of the F_2 material was included also a measurement of the length of flower for each individual. The flowers in *Lamium* vary somewhat in size according to their position on the plant and the time in the flowering period of the plant; for the rightest possible measurement as between the plants the biggest flowers on each plant at the time of the measurements were used, their average length being given to within 0,5 mm. The pollen from the measured flowers was also examined in respect of its content of filled grains. The flowers varied considerably as between the plants in respect of length; in the F_2 from $h21 \times p30$ they varied between 8 mm and 19 mm. The average length was 15,9 mm.

In part, of course, this variation may be ascribed to the different length of the flowers of the parent species; these are 18—19 mm in $p30$ but only 15—16 mm in $h21$. But a closer investigation of the offspring mentioned, which comprised a greater number of individuals than any other of the F_2 progenies, has shown the existence of a strong correlation between size of flower and pollen fertility. As this might be suspected of being in part a consequence of the effect upon the pollen fertility exercised by the chromosome number, and in part the result of an effect of the chromosome number and perhaps also of the vigour upon the size of flower, some calculations of conceivable correlations were carried out to elucidate this further; these are summarized in Table 20.

From this it appears that a significant positive correlation existed be-

TABLE 20. *Some coefficients of correlations and of partial correlations between different characteristics in the F_2 from h21×p30 (n=75).*

Correlations:	r
Size of flower — pollen fertility	+0,580 ***
Size of flower — chromosome number	+0,273 *
Pollen fertility — chromosome number	+0,414 ***
Size of flower — vigour of plant	+0,294 *
Chromosome number — vigour of plant	—0,138
Pollen fertility — vigour of plant	+0,168
Size of flower — vigour of plant when chromosome number is eliminated	+0,338 **
Size of flower — chromosome number when vigour of plant is eliminated	+0,320 **
Size of flower — pollen fertility when chromosome number is eliminated	+0,535 ***
Size of flower — pollen fertility when chromosome number and vigour of plant are eliminated	+0,501 ***

tween the size of flower on the one hand and the chromosome number and vigour, respectively, on the other hand when the two latter factors were alternately eliminated. As has been shown above, there was also a significant positive correlation between the pollen fertility and the chromosome number, as well as a faintly positive one between the pollen fertility and the plant vigour. If, however, the effects of both the chromosome number and the vigour of the plant are eliminated, there remains a marked and significant correlation between the size of flower and the pollen fertility.

The correlation shown may possibly imply linkage of factors for size of flower and factors causing sterility in the hybrids. The flowers of the F_1 hybrids were certainly intermediate in size between those of the parent lines, and did not show any more pronounced tendency than that characterizing *h*21 to stunt the size of flower at the end of the flowering, or in the autumn. Nor was there any considerable difference in the size of flower as between the parents. If, however, the flowers of the species which contributed the unpaired genome to the F_1 hybrids were very small (*e. g.*, for the most part cleistogamic), a correlation like that shown might be conceived to arise inasmuch as the chromosomes from the unpaired genome were in different degrees more represented among the F_2 plants than in the F_1. Such an interpretation is but little supported by the morphological observations on the F_2. One must expect, on the

other hand, that the chromosomes of the odd genome were, more than the others, under-represented in the F_2, chiefly as a result of the elimination of univalents. Such a condition must prevent a correlation like that demonstrated.

Investigations of possible interrelationships between interspecific differences and hybrid sterility have been carried out on different plant materials. Of five cases reviewed by STEBBINS (1950), three showed independence of morphological divergence and the development of hybrid sterility; two showed such an association in consequence of genetic linkage. Furthermore, in backcrosses with the parents of hybrids between *Nicotiana Langsdorffii*×*N. Sanderae*, SMITH (1952) has found that the more parent-like plants probably had a somewhat better pollen fertility than the others; no such correlation was traceable in the F_2 hybrids.

As regards association between size of flower and degree of fertility, however, chromosomally aberrant and therefore partially sterile plants in especially *L. intermedium* (*e. g.*, BERNSTRÖM, 1953 b, pp. 415—416), *L. hybridum* and tetraploid *L. amplexicaule* have several times proved also to have smaller flowers than the normal. A phenomenon closely related to this may possibly be that tetraploid *L. amplexicaule* has, relatively speaking, significantly fewer chasmogamic flowers than diploid, and that the aneuploids frequently have far fewer chasmogamic flowers than the euploids (BERNSTRÖM, 1950 b, 1954). Similarly, STEBBINS (1949) has found that autotetraploid *Bromus catharticus* is almost entirely without chasmogamic flowers with their large anthers, which flowers appear in the diploids at the height of the flowering season under particularly favourable conditions of moisture and soil. STEBBINS is inclined to associate the absence of chasmogamic flowers with the poor fertility of the tetraploids.

In the F_2 after *Galeopsis ladanum*×*G. ochroleuca*, MÜNTZING (1930) found a positive correlation between the size of anthers and length of the flowers. The smallest anthers were shrivelled. MÜNTZING assumes a bifactorial explanation of the difference in size of the anthers, but as the hybrids were partially pollen sterile, a continuous variation in sterility would seem likely, reminiscent of that under consideration in *Lamium*. There are in *Lamium* also some other cases in which the size of flower and the fertility seem as if they might stand in a positive relation to each other; this applies especially to the F_1 hybrids *L. hybridum*×diploid *L. amplexicaule*, tetraploid *L. purpureum*×diploid *L. amplexicaule* and *L. purpureum*×*L. bifidum* subsp. *balcanicum*.

These all have considerably smaller flowers than the parents, while their allopolyploids have flowers so much larger — and well developed — that it does not seem possible to ascribe the increase in size to the doubling of the chromosome number alone; rather does it seem as if it might be connected with the simultaneous considerable improvements in the fertility, these thus exerting a favourable influence *in et per se* upon the development of the flowers. The vigour of the plants was perfectly good even at the diploid and triploid stages.

When discussing the pollen fertility of the dwarfish hybrids between *L. hybridum* and tetraploid *L. purpureum* it was remarked that this is strongly correlated with the size of the flowers, which is in its turn clearly dependent upon the vegetative development of the plants (pp. 17 and 25). The pollen sterility as estimated morphologically is thus in these hybrids in part diplontic. This is, moreover, probably the case also in the circumstances discussed above, though presumably to a far lesser extent than in the extreme cases among the dwarfish hybrids; and whereas the degree of pollen fertility in these is quite obviously dependent upon the vegetative development of the plants, the pollen fertility in the other above-mentioned cases in *Lamium* may seem to be a contributory factor in the determination of the size of flower. The plant vigour may perhaps have been of importance for the size of flower also in some of these cases (*L. intermedium*, *L. hybridum*, *L. amplexicaule*), but in the others evidently not. It will not be possible, however, to establish the cause of the correlation between size of flower and pollen fertility among the F_2 hybrids between *L. hybridum* and tetraploid *L. purpureum* without closer cytologic and genetic investigations.

2. F_2 *from dwarfish hybrids*

During 1945—2 an F_2 progeny comprising 26 plants from $h21 \times p7$ was studied. This F_2 generation was in external appearance strongly reminiscent of progenies after normally developed F_1 hybrids, and thus showed the same high degree and the same kind of morphological variation; and interestingly enough, the majority of the plants were of clearly »normal» type, without a trace of the parents' dwarf characters. Nineteen plants were with the same evidence referable to the normal type of growth as six belonged to the dwarf type; only one plant was intermediate.

The chromosome numbers varied for the entire progeny between $2n=29$ and $2n=54$, thus the same extremes as for the above-discussed

F_2 generations. Only three plants ($2n=41$, 53 and 54), however, had more than 39 chromosomes. Not counting the hexaploid plants, the average number was 36,0. For the seven dwarf plants it was 36,4 and for the remainder 35,8.

It is interesting to note that in the F_2 after dwarf hybrids a great preponderance of normally developed plants was obtained. The above-described attempts to elucidate the causes of dwarfish growth (p. 20, and discussion) have shown that such growth is probably due to very few genes, perhaps only to two complementary ones, and that it is recessive to normal growth. Two causes of the occurrence of the normally developed plants in the F_2 are then conceivable. The one is that an extensive cross-fertilization of the F_1 plants had been effected by insects from lines of tetraploid *L. purpureum*, whose F_1 hybrids with *L. hybridum* show normal growth. This, however, was in reality excluded for several reasons.

The second cause may be elimination of the chromosome or chromosomes bearing the genes responsible for the dwarfish growth in the F_1. I M in the F_1 is, as we have seen, very irregular, and loss of chromosomes in the F_2 doubtless very extensive, as is shown, moreover, by the irregular chromosome numbers, often < 36. If dwarfish growth is caused by one gene — or group of coupled genes — from each of the genomes entering the F_1 hybrids, all that is required for the inhibition of the dwarfish growth is the loss of at most both chromosomes in question from the one parent species. If *L. hybridum* has only one complementary gene for dwarfish growth, belonging to the genome which is inhomologous with those of *L. purpureum*, the loss of this single chromosome will be enough for the inhibition of the dwarfish growth.

Without a more reliable knowledge of either the genic background of the dwarfish growth or the chromosomal composition of the F_2, which can only be roughly assumed, no more accurate testing of the assumption is possible. This must, however, be said to bear in a high degree the stamp of probability; and if it is really true it is an indication, like the crossing experiments, that the genes for dwarfish growth must be very few, inasmuch as there was only one intermediate plant in the F_1, while the rest clearly belonged to normally developed hybrids or dwarfs; it confirms, moreover, the earlier conclusion that dwarfish growth in the F_1 cannot be a cytoplasmic effect, but must be purely gene-conditioned.

Finally, it may be mentioned that three of the plants in the above-described F_2 from $h21 \times p30$ were quite clearly of dwarf type, and that also one plant in $h36 \times p30$ was of the same kind. Although the dwarf

plants in these progenies were not expected, there could be no doubt, from the genetic point of view, of their nature, so characteristic is the appearance distinguishing the type of dwarf plants. The occurrence of these four dwarf plants confirmed the assumptions made earlier on other grounds that *p*30 had not been pure, but had been cross-fertilized with *p*6 or *p*7 (*cf.* above, and BERNSTRÖM, 1954). The dwarfs were evidently able to appear in the F_2 after chromosomal elimination of the dominant gene or genes for normal growth. In the F_2 from *h*21×*p*42 and *h*36×*p*42 not a single dwarf plant occurred. For various practical reasons it was altogether out of the question that the dwarfs in the F_2 should have been able to appear through spontaneous cross-fertilization of the F_1 hybrids with such lines of *L. purpureum* as, crossed with *L. hybridum*, produce dwarfish growth, or with hybrids in which such lines entered.

C. Investigations on F_2 generations from L. intermedium×tetraploid L. amplexicaule

Altogether 44 F_2 hybrids, cultivated in three different plant generations and descended from *i*22×*a*14 and *i*39×*a*14, respectively, have been studied. On account of *L. intermedium*'s homogeneity, the two kinds of progeny may be regarded as genetically equivalent in a very high degree (*cf.* BERNSTRÖM, 1953 b).

Cytogenetically, these F_2 progenies might be expected to form a close parallel to those after *L. hybridum*×tetraploid *L. purpureum*, and this has also been found to be the case. The chromosome numbers varied between $2n=34$ and $2n=43$, and the total distribution showed good agreement with that in the above-mentioned hybrids. Also the average seed-setting, the pollen fertility and the vigour were of the same order of magnitude as in these. In the biggest of the progenies, comprising 18 plants, the correlation coefficient for the correlation between chromosome number and height of plant was $-0{,}201$ ($0{,}5 > P > 0{,}4$); the plant vigour was not assessed in this year. The correlation coefficient obtained indicates that decreasing height of plant with increasing chromosome numbers may also characterize these hybrids.

The F_2 plants varied from very stout to extremely slender and thin individuals; in their external appearance, however, the majority of the plants were more or less clearly reminiscent of the F_1 hybrids. None of the plants even approximately resembled either of the parent species, and still less any other species than these. No plants with such clearly dimorphic flowers as those of *L. amplexicaule* were found.

DISCUSSION

I. Cross compatibility and incompatibility

In order to give to the reader a survey of the hybrids which it has been possible to produce, and also of the cross combinations which it has not been possible to realize, the scheme in Fig. 37 has been drawn up. Unrealizable cross combinations not entered in the scheme for the sake of general clarity are mentioned in the explanation to the figure. For further details concerning the crossing experiments the reader is referred to an earlier paper (BERNSTRÖM, 1953 a) as well as to the data in the present paper. *L. purpureo-bifidum* was not crossed with *L. amplexicaule* owing to lack of chasmogamic flowers in the latter at the time when the attempt was to be made. The letters used to designate the genomes will be discussed below.

In the above-cited paper it was pointed out that in *Lamium* the chromosome number in the components of various cross combinations had proved decisive for the compatibility, inasmuch as no combination had been found in which it had been possible to produce hybrids with different multiples of the chromosome number by changing the chromosome number in the one parent or in both of the parents, even when these were autotetraploids. This has been tried in six combinations, including four species and one allohexaploid (*cf.* Fig. 37). Since in two cases it is triploid hybrids and in one case a pentaploid that it has been possible to produce, it is here not in consequence of changed relations between the chromosome numbers in the embryo, endosperm and mother-tissue as compared with the normal ones that the crossings are a failure, as suggested by WATKINS (1932) and MÜNTZING (1933). Three different combinations between allohexaploids and diploid species have been tried with the aim of avoiding incompatible combinations between tetraploids, which would have resulted in hybrids with corresponding combinations of genomes, but without success. In one of the cases, *L. purpureo-amplexicaule* × diploid *L. amplexicaule*, the combination was unsuccessful despite the fact that the embryos got the same constitution as *L. intermedium*, as would also, moreover, have been the case in hybridization between the autotetraploids of *L. purpureum* and *L. amplexicaule*. Similarly, it is not possible, either, to produce hybrids between *L. intermedium* and diploid *L. purpureum*, although the embryos get exactly the same constitution as do those between tetraploid *L. purpureum* and diploid *L. amplexicaule* which are able to develop. It is

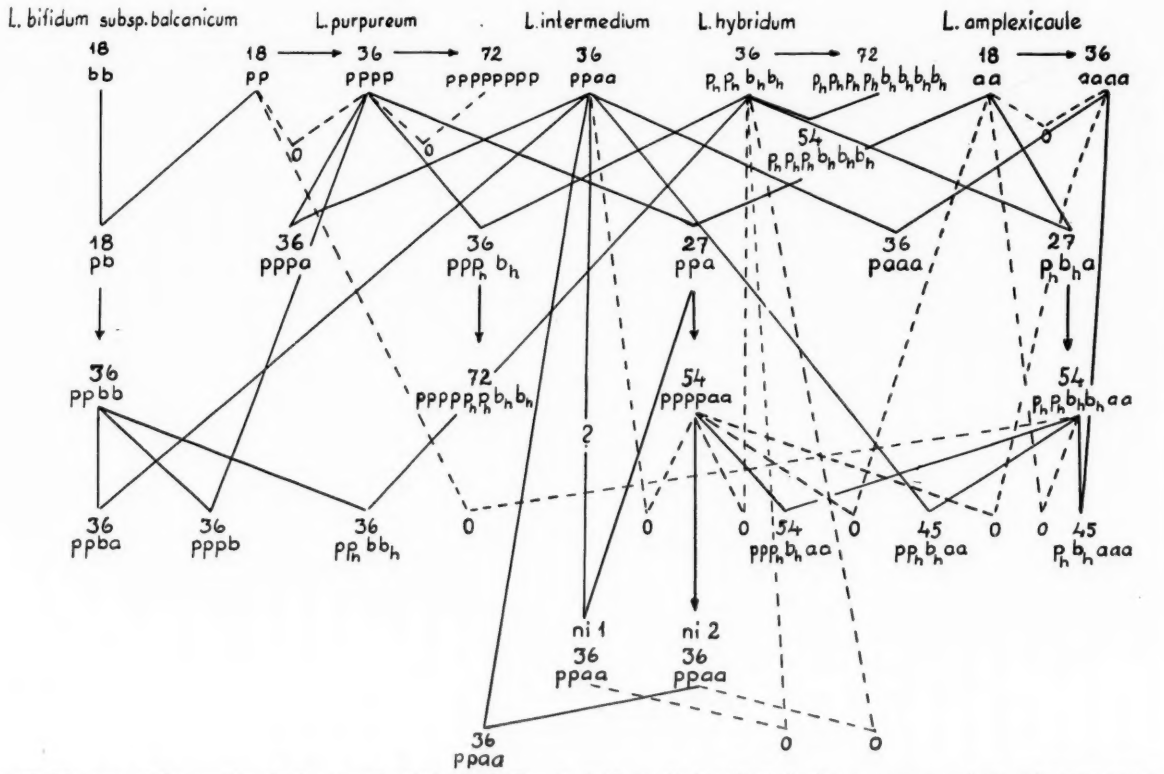


Fig. 37. Diagram of the species (incl. autopolyploids) and allopolyploids studied, with their chromosome numbers and genome symbols. Successful crossings are indicated by solid lines, and unsuccessful attempts at crossing, by broken lines. For the sake of surveyability, all cross combinations between species (except *L. bifidum* subsp. *balcanicum*) have been omitted from the uppermost level of the diagram, which comprised diploid and/or tetraploid forms, and which have failed so far.

worth pointing out that the cross experiments between *L. purpureo-amplexicaule* and diploid *L. amplexicaule* included also the best fertile line of the former and the line of *L. amplexicaule* entering this line.

It thus appears that the crossability between the species of *Lamium* is regulated by both quantitative and qualitative factors, and that the constitution of the resulting embryos is, at least in certain cases, not to blame for the incompatibility between the two parties. On account of the general unpredictability as concerns the crossability between the different species, *etc.*, one may assume that the qualitative factors of importance for the crossability may, as a rule, be referred to several genes in the respective genomes which regulate the processes having to do with the fertilization and the embryonal development. This genetic differentiation begins already within the species. In the above-cited paper, for example, several instances from *Lamium* were adduced to show the dependence of interspecific crossability upon interlinear genetic differences. From the present paper it may be mentioned that it was easily possible to hybridize *p*53 with *L. bifidum* subsp. *balcanicum*, but *p*29 — not at all. This instance is of particular interest, since there is a possibility that the difference in crossability is due to a simple gene difference between the two lines. It has been shown above that dwarfish growth in hybrids between *L. hybridum* and tetraploid *L. purpureum* is probably due to one gene in the latter in conjunction with one or several genes in *L. hybridum*. *p*29 has this gene, while *p*53 lacks it. We shall revert to this question later. An example of an in its effect similar, interlinear divergence also in *L. hybridum* is constituted by the inviable embryos in hybrids between *h*89 and *L. purpureo-bifidum*, as compared with the well-developed seeds of the latter's hybrids with *h*37 and *h*49.

As more or less pronounced dwarfish growth has sometimes characterized hybrids between different species and lines within the genus (of which more below), it is reasonable to suspect embryo lethality through a complementary gene effect genetically related to that producing the dwarfish growth as the cause of incompatibility, as was the case above for *p*29×*L. bifidum* subsp. *balcanicum*. Such a gene effect has been noted by several authors (*e. g.*, EAST, 1935; MELCHERS, 1939; SEARS, 1944; GERSTEL, 1954). Apart from the possible occurrence of the effect in the above-mentioned case, which is very uncertain, it has not, however, been possible, as far as the investigations have extended, to trace any such cause of a crossing failure, though this does not, of course, exclude the possibility of its occurrence.

In recent years many reviews have been published of investigations

and experience concerning crossability within thoroughly investigated genera, including both diploids and polyploids, which have been able to throw light upon various aspects of the subject and draw attention to different factors which are undoubtedly of importance for the crossability between species (GOODSPEED, 1945; LAMM, 1945; BRINK and COOPER, 1947; NORDENSKIÖLD, 1945; and others). The many-sidedness of the subject has thus been well elucidated, and the unpredictability of crossability has become more understandable; but it has also become obvious that the gaps in our knowledge are still great, and that not only more thorough investigations than those which have in general been performed hitherto, but also new methods are needed really to throw new light on these problems (*cf.*, *e. g.*, HALL, 1954). Here, I will only observe that the crossability between the different species of *Lamium* has been able to afford just as little guidance concerning the relationships between them, as has frequently been the case also in other plant genera comprising diploids as well as polyploids.

In a previous paper, finally, attention was drawn to the remarkable circumstance that *L. intermedium* is an allotetraploid between *L. purpureum* and *L. amplexicaule*, which have not, despite repeated attempts, proved crossable at all as diploids and which are both, moreover, in a high degree autogamic (BERNSTRÖM, 1953 b). If one abstracts from the possibility that an autotetraploid form of either one of the parent species may perhaps occur somewhere, though hitherto undiscovered, with whose help it has been possible for *L. intermedium* to originate *via* a triploid hybrid, one must imagine that it has been possible for this species to originate thanks to a conjuncture of favourable circumstances. An unreduced gamete in either species may have been causative; no such gametes have ever been observed, however, in either the one or the other. It has proved that the crossability may be very different between tetraploid *L. purpureum* and diploid *L. amplexicaule*, according to the choice of line in both species (BERNSTRÖM, 1953 a). It is then natural to assume that on crossing different diploid lines of these the development of the embryo may proceed to different stages before it stops for some reason as yet unknown. Possibly a few lines in each of the species are in this way really crossable under normal conditions of growth.

Perhaps extreme climatic and ecological conditions having, amongst other things, a strong influence upon the osmotic pressure of the plants are needed for a crossing to succeed between the nearest compatible lines of either species (*cf.* SCHLÖSSER, 1936). The crossability within *Lamium* has as a matter of fact proved to be very clearly favoured by

extremely warm and dry conditions of growth. Besides by nectarivorous Hymenoptera (BERNSTRÖM, 1952), the flowers of both species are visited by, *inter alia*, butterflies of the genus *Pieris* (SELANDER and BRYANT-MEISNER, 1909), which perhaps increases the possibilities of cross-pollination. Reasons have earlier been adduced to show that cross-fertilization regularly but rarely takes place in *L. amplexicaule* (BERNSTRÖM, 1952), and as both species frequently occur in the same kind of locality, cross-pollination must also be conceived to occur regularly between them. Complete self-fertilization probably scarcely exists among phanerogamic species.

II. Relationships between the species primarily as indicated by the degree of homology between their genomes

In Fig. 37 the genomes entering for each species, *etc.* have been noted. A letter corresponds to one genome. In the account of the species hybrids above it was pointed out, in connection with the cytological investigations, what conclusions concerning the relationships between the respective species the chromosome pairing in their hybrids entails. Here a summary will be given with reference to Fig. 37.

The nature of the genomes entering in *L. intermedium* is shown by the appearance of two new lines of *L. intermedium* in the cultures from hybrids between *L. purpureum* and *L. amplexicaule*. The hybrids between tetraploid *L. purpureum* and diploid *L. amplexicaule* have in I M almost exclusively bivalents and univalents. More than 3 % of the PMC's have evidently one to two heterogenetic configurations, and one must then expect that also some of the cells with apparently exclusive homogenetic pairing do actually conceal occasional bivalents from heterogenetic pairing. The conclusion, however, is that between the genomes of *L. purpureum* and *L. amplexicaule* there is only a slight degree of structural resemblance. The hybrids between *L. intermedium* and tetraploid *L. purpureum* and between *L. intermedium* and tetraploid *L. amplexicaule* may consequently by expected to have predominantly trivalents (or bivalents+univalents) and univalents in I M. The investigations above have shown, moreover, that this is the case.

In the presence of three homologous genomes and one strongly inhomologous genome in these hybrids the frequency of trivalents may appear surprisingly high; as has been described in detail above, it is indicated by the small number of univalents, which were most often only nine, in relation to the number of configurations. The autotetra-

ploids of *L. purpureum* and *L. amplexicaule,* however, had about six quadrivalents+trivalents per cell, out of nine possible; as compared with various species investigated by different authors this frequency seems to be well over average of the observed frequency in relation to the greatest conceivable one. Since, further, somewhat fewer chiasmata per chromosome are required on an average for trivalents than for quadrivalents, at the same time as for the chiasma formation there are probably also greater mechanical difficulties in connection with a larger number of chromosomes, one may expect that the frequency of trivalents per cell will in an autotriploid be somewhat higher than the frequency of quadrivalents in the corresponding autotetraploid; this agrees, moreover, with actual experience (*cf.* UPCOTT, 1935; DARLINGTON, 1937). Syndetically, the hybrids between *L. intermedium* and tetraploid *L. purpureum* and *L. amplexicaule*, respectively, have much in common with autotriploids as regards the chromosomes of the two last-mentioned species, although the presence of an inhomologous genome probably has rather a disturbing effect, perhaps through the higher chromosome number *in et per se*, and certainly through the by all means low degree of structural homology between the species. — As other instances of high frequencies of trivalents it may be mentioned that in triploid maize the chromosomes were very often associated in ten trivalents, although 9 III+1 II+1 I was most common (RANDOLPH and MCCLINTOCK, 1926; MCCLINTOCK, 1929). In a triploid plant of *Lolium perenne* 41,5 % of the PMC's had 7 III, 32,3 % had 6 III and 19,5 % had 5 III, *etc.* (MYERS, 1944).

In this connection it should also be borne in mind that high temperature and a good supply of nutriment may each severally be able to contribute to a high chiasma frequency (*cf.* WICKBOM, 1945; GRANT, 1952). The experimental material was, certainly, cultivated in pots in a greenhouse and kept well supplied in the respects mentioned.

It is remarkable that the cells with 8 I+9 configurations in the euploid plant of *L. intermedium*×tetraploid *L. purpureum* (Table 1) are five out of in all 60, *i. e.*, 8 %, just as correspondingly in *L. intermedium*×tetraploid *L. amplexicaule* the same kind of cells constitute 11 % (Table 2). Cells with such pairing must, as has been pointed out earlier, have one quadrivalent, whose appearance may primarily be assumed to be due to partial homology between the *L. purpureum* and *L. amplexicaule* genomes. In the hybrids between tetraploid *L. purpureum* and diploid *L. amplexicaule* the trivalents correspond to this quadrivalent-formation. In the triploid hybrids, however, one would

expect an at least equally high frequency of trivalents as of cells with nine configurations + 8 I in the tetraploid hybrids; the frequency in the latter is instead many times higher.

This difference may be explained by the assumption that the two kinds of genome entering in *L. intermedium* have a chromosomal structure somewhat different to that in the pure species. It is, certainly, very probable that the line of *L. amplexicaule* which formed *L. intermedium* was structurally different to that entering in the cytologically studied hybrid between these species; such differences are common between lines of *L. amplexicaule*, though they are generally not so considerable (BERNSTRÖM, 1952). It also appears to be a very likely assumption, however, that *L. intermedium* originated from a diploid or triploid hybrid through the functioning of ± diploid gametes, whose appearance was preceded by the formation of one or several heterogenetic bivalents, with structural recombinations between the genomes as a consequence. If the hybrid was diploid, the degree of heterogenetic pairing was probably more extensive than in the triploid hybrids studied. Thus in this way *L. intermedium* may be conceived as originating from a tetraploid, more or less structurally heterozygotic plant, and not as entirely composed of pure *L. purpureum* and *L. amplexicaule* genomes.

Such an explanation of the cytological incongruities found is supported by the observation that spontaneously arising, strict allopolyploids have in the great majority of cases originated through the functioning of unreduced gametes and not through somatic doubling of the chromosome number in the F_1 hybrids (CLAUSEN, KECK and HIESEY, 1945). Chromosome-structural recombinations between the two kinds of genome even after the appearance of *L. intermedium* as a result of occasional heterogenetic associations are less likely to have occurred on account of the chromosomal homogeneity of *L. intermedium* (*cf.* BERNSTRÖM, 1953 b).

If it has been possible to solve in its main lines the problem of the origin and nature of *L. intermedium* this is, on the other hand, far from being the case as regards *L. hybridum*. The chromosome pairing in I M in the hybrids of this species with tetraploid *L. purpureum* and diploid *L. amplexicaule*, respectively, has shown that *L. hybridum* must be an allotetraploid, inasmuch as the pairing in both kinds of hybrids takes place between only one genome from *L. hybridum* with the genomes from each of the other two species. The preponderant bivalent formation in the hybrids between *L. hybridum* and *L. purpureo-bifidum* likewise shows that the former is an allotetraploid. Further, the cytological in-

vestigations have demonstrated that *L. amplexicaule* can obviously not be the one of the parent species of *L. hybridum*. If this had been the case, the chromosomes that in the triploid hybrids chiefly form bivalents (BERNSTRÖM, 1952) might in the hexaploid hybrids have been expected to form multivalents in far greater numbers than was the case (Table 9 and p. 43). Instead, bivalents were formed quite predominantly on account of differential affinity (DARLINGTON, 1937; *cf.* STEBBINS, 1945). The two genomes which associate in the triploids are thus evidently only partially homologous. Indications of this are, moreover, the rather varying pairing conditions between the pollen mother cells which characterize the triploid hybrids and the marked differences in the average occurrence of especially multivalents which characterize the hybrids of various *L. amplexicaule* lines with *L. hybridum*, and of which the causes do not appear or appear only to a slight extent in intraspecific hybrids between these same lines of *L. amplexicaule* (BERNSTRÖM, 1952).

The genome from *L. hybridum* which in triploid hybrids with *L. amplexicaule* associates with the chromosomes from the latter can scarcely be the same genome as pairs with the chromosomes from *L. purpureum* in the hybrids *L. hybridum*×tetraploid *L. purpureum*, for the syndesis is good in both kinds of hybrids, whereas the chromosomes from *L. purpureum* and *L. amplexicaule* are, as pointed out above, almost completely inhomologous. If the same genome from *L. hybridum* paired with the genomes of *L. purpureum* and *L. amplexicaule*, it must thus structurally occupy an intermediate position between them such that it would be capable of rather complete syndesis with them both. From a general cytological point of view it would seem extremely unlikely that this is the case. One would then scarcely expect such a common occurrence of multivalents in I M of *L. hybrido-amplexicaule* as there actually was (Table 9), and still less would one expect so few univalents and so frequent multivalents in the hybrids between *L. hybrido-amplexicaule* and *L. intermedium* (Table 12, p. 51).

Identity between the genome from *L. hybridum* which associates with the genomes of *L. amplexicaule* and *L. purpureum*, respectively, would imply, further, that the genomes in *L. bifidum* subsp. *balcanicum* were almost completely inhomologous with those in *L. purpureum* and *L. amplexicaule*. This emerges from the fact that whereas the diploid hybrids between *L. bifidum* subsp. *balcanicum* and *L. purpureum* were completely sterile, the tetraploid hybrids were decently fertile, and the chromosomes formed almost exclusively bivalents. The sterility of the di-

ploids may therefore be assumed to have been chromosomal, and due to deficient pairing between the genomes of the parent species. As the chromosome pairing in *L. hybridum*×*L. purpureo-bifidum* occurs quite predominantly in bivalents, one of the genomes from *L. hybridum* must be on the whole homologous with the genome in *L. bifidum* subsp. *balcanicum*. The genome in question in *L. hybridum* could then not be the same as that enabling the bivalent-formation in *L. hybridum*× *L. amplexicaule*, on the assumption here under discussion.

The assumption that the genome from *L. hybridum* which associates with the chromosomes from *L. amplexicaule* is instead inhomologous with those of *L. purpureum*, however, fits in with what has actually been observed far better than the above-discussed assumption. In previous chapters when dealing with the cytology of different hybrids in which *L. hybridum* enters, we have, moreover, proceeded on this latter assumption. The genome in *L. hybridum* which is partially homologous with the genome from *L. amplexicaule* will then be the same that in the hybrid *L. hybridum*×*L. purpureo-bifidum* is structurally similar or almost so to the genome of *L. bifidum*; in Fig. 37 they have been denoted b_h and b, respectively. The assumption explains the rather sparse occurrence of univalents as well as the regular occurrence of multivalents in *L. hybrido-amplexicaule*×*L. intermedium*, and the regular occurrence of multivalents in *L. hybrido-amplexicaule*, too. It also explains why the univalents in *L. hybrido-amplexicaule*×tetraploid *L. amplexicaule* were not still more numerous than was the case.

As the parent species to *L. hybridum* could not be traced by direct and unequivocal re-synthesis of this species, its genomes have in Fig. 37 been given an extra designation h in addition to the designations for genomes from other species with which the genomes of *L. hybridum* are thus evidently structurally homologous. The genome designations in Fig. 37 refer to genetically different genomes, in WINKLER's (1916) sense; dissimilar genomes may thus pair regularly, as is well exemplified by an instance like, *e. g.*, *Festuca pratensis*×*Lolium perenne* (PETO, 1933). The observations on the chromosome pairing in hybrids between *L. hybridum* and tetraploid *L. purpureum* indicate, moreover, that also minor structural differences may occur between the p_h and the p genomes. The common occurrence of cells with 17 II+2 I instead of 18 II in the hybrids between *L. hybridum* and *L. purpureo-bifidum* has in the same way, along with genetic divergences between the genomes to which we shall revert later, clearly emphasized the necessity for the

time being of giving also the other kind of genome in *L. hybridum* a special designation.

As long as it is uncertain whether the p_h genomes in *L. hybridum* are identical with the genomes of *L. purpureum* or not, it is also uncertain whether the genetic differences between *L. hybridum* and *L. purpureo-bifidum* are to be referred to differences between only the b_h and b genomes, or to differences between these as well as between the p and p_h genomes. *L. purpureo-bifidum* and *L. hybridum* differ genetically in several respects. Morphologically, the first-mentioned one diverges in several characters quite clearly from *L. hybridum*, and has thus its own characteristic appearance, even if it must also be observed that *L. purpureo-bifidum* is in many of its features reminiscent of *L. hybridum* and is taxonomically obviously closely related to the latter. *L. purpureo-bifidum* is furthermore crossable with *L. intermedium*, which *L. hybridum* is not (MÜNTZING, 1926; JÖRGENSEN, 1927; BERNSTRÖM, 1953 a). The F_1 hybrids between *L. purpureo-bifidum* and *p*60 were slightly disharmonious, while those with *p*61 were almost normally developed; the corresponding hybrids with *L. hybridum*, on the other hand, were normally developed and dwarfs, respectively. The hybrids themselves between *L. hybridum* and *L. purpureo-bifidum* did not reach the size of their parents, and in the F_2 dwarfs were segregated. These and other cases of dwarfish growth will be discussed in greater detail below.

Finally, the F_1 and F_2 hybrids between *L. hybridum* and *L. purpureo-bifidum* were characterized by a slight though fully significant pollen sterility, which has never been found in intraspecific hybrids of *L. hybridum*. The pollen sterility is probably connected with the fact that 37 % of the PMC's in the F_1 hybrids had two to four univalents, as compared with 4 to 5 % in the parents; the percentages must be taken with a certain reservation, as the fixations were made on different occasions. The common occurrence of univalents may conceivably be due to the minor chromosome-structural differences between the p and the p_h genomes discussed above (p. 14). It may also be due to differences between b and b_h. Trivalents seem never to occur in *L. hybridum*, though they do in *L. purpureo-bifidum*.

In assessing the relationship between *L. hybridum* and *L. purpureo-bifidum* one must bear in mind several circumstances. In an earlier paper it has been pointed out that *L. hybridum* — as far as is shown by the experimentally investigated material — may rather be assumed to have originated on a single occasion a long time ago, than on repeated occasions (BERNSTRÖM, 1953b). This assumption was based chiefly upon

two circumstances. The one was that all intraspecific F_1 hybrids comprising 10 different lines and 13 combinations had been fully fertile; the lines had relatively very different appearances and derived from widely separated parts of the area of distribution of the species. Although more lines and more hybrid combinations between the lines would certainly have been desirable, the good fertility found throughout in the hybrids must be taken as a strong indication that at least for the most part *L. hybridum* has the same chromosomal structure. The second circumstance was the signs of structural differences between p and p_h. In view of the fact that *L. purpureum*, more surely than *L. hybridum*, appears to be chromosome-structurally homogeneous (BERNSTRÖM, 1953 b), at the same time as it must on account of its systematic and also cytological position be strongly suspected of being one of the species from which *L. hybridum* has originated, this might imply structural recombinations arisen in connection with the origination of this species.

CLAUSEN, KECK and HIESEY (1945) have observed that the great majority of spontaneously arisen allotetraploids have appeared through the functioning of unreduced gametes formed by F_1 hybrids between the parent species. There is no reason to assume that *L. hybridum* either originated in any other way; and if we assume that the species originated on several occasions it must also follow, in consideration of *L. hybridum*'s cytological homogeneity, firstly that the different parent lines of the two species have always had quite the same chromosome-structural constitution, and secondly that no chromosome pairing, either autosyndetic or allosyndetic (*sensu* LJUNGDAHL), or chiasma formation preceded the formation of the unreduced gametes.

The majority of diploid hybrids of inter-cenospecific nature (*loc. cit.*) that have given rise to true-breeding allopolyploids have been characterized by a large or even complete absence of bivalents in I M. The highly regular bivalent formation in *L. hybridum* and the always complete fertility of its interlinear hybrids indicate that the two kinds of genome in the species are very inhomologous; one would otherwise have expected structural recombinations in consequence of heterogenetic pairing, resulting in lines with another chromosomal structure than the original one. One must therefore for the time being assume that the meiosis in the diploid hybrid or hybrids giving rise to *L. hybridum* was also characterized by very limited chromosome pairing. It is thus well within the reach of possibility that no chromosome pairing in the pollen mother cells to the unreduced gametes which may be presumed to have given rise to *L. hybridum* once and perhaps several times has taken

place; and even if this was the case the new chromosomes may have been eliminated in subsequent generations just as well as they may, on the other hand, have been stabilized or have given rise to other types again.

If the pairing conditions in *L. hybridum*×tetraploid *L. purpureum* which may indicate structural divergences between p and p_h are actually based only upon a partial homology between p and b_h, so that a special p_h genome does not exist, then the question arises of how chromosome-structurally uniform the parent species of *L. hybridum* may be. Of those chiefly suspected — and any other known species than *L. purpureum* and *L. bifidum* are scarcely available —, the question is already answered as far as *L. purpureum* is concerned. The structural stability of this species may furthermore be presumed to have been established for a very long time (BERNSTRÖM, 1953 b). Chromosome-structurally uniform species have in the light of investigations by, *e. g.*, SAX (1933) and GAJEWSKI (1953) proved to be able to retain their homogeneity over very long periods. Just how uniform from the cytological point of view *L. bifidum* may be, on the other hand, we do not know, any more than we know the degree of its morphological variability. None of the more important floras from the Balkans and Italy has anything to tell us of any morphological variation within the species, including subsp. *balcanicum* (HAYEK and MARKGRAF, 1931; VELENOVSKY, 1893; STOYANOFF and STEFANOFF, 1925; FIORI, 1925—29). Unless a possible variation were very pronounced, however, one could scarcely expect its existence to be drawn attention to in such works. Studies of herbarium materials from Lund, Upsala, Stockholm, Gothenburg, Copenhagen and Helsinki have not been able to reveal any considerable variation.

The circumstance that *L. bifidum* occurs in a main form and a subspecies, subsp. *balcanicum*, shows that it has undergone processes of differentiation; and as such processes have taken place, they may also conceivably have resulted in other forms, too, which have now disappeared or occur as remnants. Taxonomically, *L. bifidum* with subsp. *balcanicum* is in many ways more related to *L. amplexicaule* than to *L. purpureum*, through the bimorphism of the flowers, the shape of the chasmogamic flowers, the lobed shape of the leaves, the slender and long-branched type of growth, *etc.* Its systematic position in the genus leaves room for the suspicion that it may be at least partly responsible for the morphological and cytological variation characterizing *L. amplexicaule*, through hybridization of these species (BERNSTRÖM, 1952). If this has been the case, then also *L. bifidum* must be presumed to

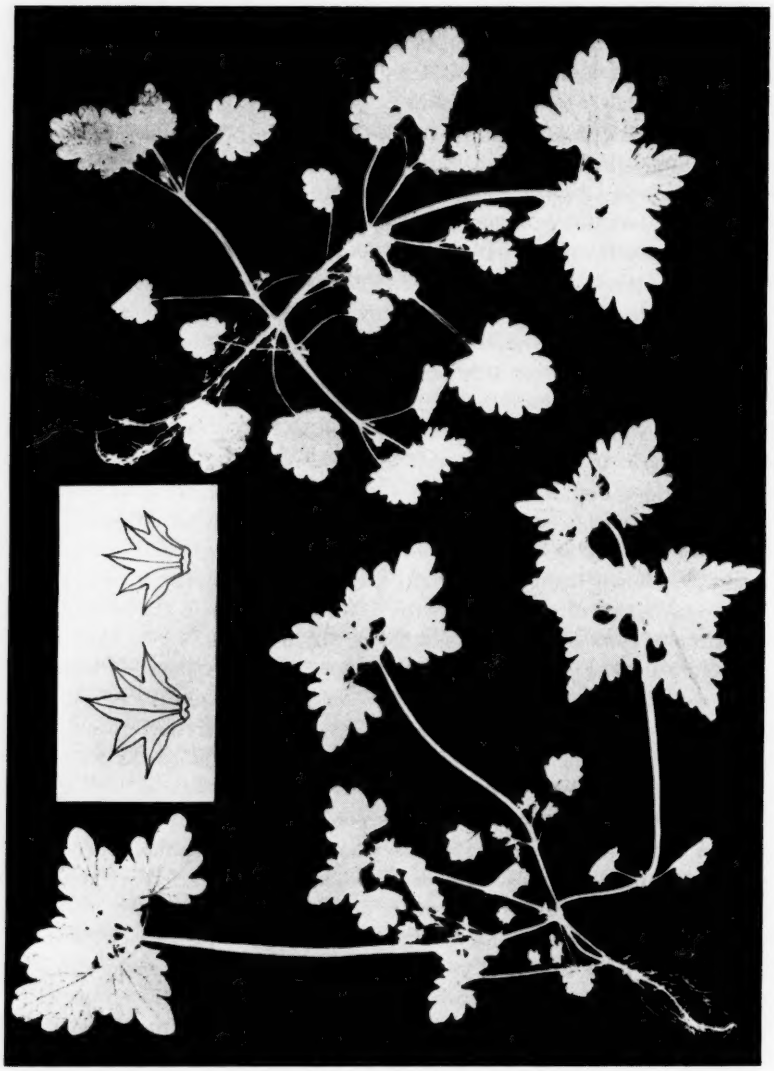


Fig. 38. *L. hybridum* (above) and *L. bifidum*. The plants were cultivated contemporaneously in the spring, the former having been sown late in the winter in greenhouse, while *L. bifidum* had overwintered in the greenhouse. The calyces belong to *L. bifidum*. — × $^1/_2$.

show a similar intraspecific variation. And if this is so, then, in reason, lines of *L. hybridum* of different origin ought to be chromosome-structurally different.

If *L. bifidum* with subsp. *balcanicum* is genetically variable, then in view of the several hereditary differences it has been possible to establish between *L. hybridum* and *L. purpureo-bifidum* it is conceivable that if the former has originated only once, a line of *L. bifidum* or subsp. *balcanicum* differing considerably from that used in the experiments may have entered; if on the other hand *L. hybridum* has originated several times, then it is for both cytological and genetic reasons not very likely that *L. bifidum* with its subspecies has a part in this. One must, however, also reckon with the possibility that a variety of *L. purpureum* genotypically rather different from the line entering in *L. purpureo-bifidum* may enter in *L. hybridum* and contribute to the evident difference between the latter and *L. purpureo-bifidum*. With our present deficient knowledge of *L. bifidum* as well as of other species and varieties of *Lamium* occurring in south-eastern Europe and south-western Asia it is impossible to infer anything more as to the nature and origin of *L. hybridum* than has been done above.

III. HYBRID DWARFISHNESS WITHIN LAMIUM

Data have been adduced in the foregoing (pp. 20 ff.) which seem to support the assumption that a single gene in certain lines of *L. purpureum* may produce dwarfish growth in hybrids with *L. hybridum*. Several cases of dwarfish growth or other developmental inhibitions even to the point of embryonic lethality are known from species crosses in plants, as also from intraspecific crosses between races or varieties. The genetic basis of developmental disturbances may probably be more or less complex, *e. g.*, in several species combinations within *Nicotiana* (KOSTOFF, 1941—43). In other cases the genic cause is clear, and of a simple nature. In the well-known case in *Crepis*, described by HOLLINGSHEAD (1930), F_1 hybrids between *C. capillaris* and *C. tectorum* either developed normally or died at an early stage of development, according to which line of *C. tectorum* was used. The lethality was caused by a single gene in this species, and independently of which line of *C. capillaris* entered. The case thus reminds one of the conditions found in *Lamium*, even in the point that all the investigated lines of the one species carry the genes or gene injurious for the hybrids.

In cotton two rather similar, misshaped and poorly viable types of F_1

hybrid, »crumpled» and »corky», have been studied (HUTCHINSON, 1932; SILOW, 1941; STEPHENS, 1946, 1950). The first-mentioned type may appear in connection with intraspecific hybridization within *Gossypium arboreum*, and also in connection with interspecific hybridization, while the other type may occur in combinations with certain lines of *G. barbadense* and *G. hirsutum*. In both cases the defects in the hybrids are caused by two complementary genes, of perhaps complex nature, which occur in certain lines of the respective species, but not in others; in the absence of one or both, vigorous hybrids result, and when purely bred they have no injurious effect.

In *Godetia Whitneyi*, HIORTH (1942) has obtained results resembling the conditions in *Gossypium*, though here it is a matter of crossings merely between races, and the effect was, as a rule, lethality at the cotyledon stage. This was produced by a gene occurring only in some of the plants, and in the carriers it is without visible effect; only in certain racial hybrids does it have the effect described. In an autogamic species like barley, WIEBE (1934) has likewise found a lethal effect at a young stage in the F_1 hybrids between two special lines through the complementary action of two otherwise unnoticed and harmless genes. Many more examples of the same kind as those described above might be given (*cf.*, *e. g.*, SEARS, 1944; GERSTEL, 1954).

The complementary genes or groups of genes in the above examples all have the feature in common that they prevent the hybrids from reproducing by checking their development at an early stage. Several authors draw attention to the importance this may have for an effective prevention of gene exchange between otherwise crossable individuals or varieties, and point out that this in its turn may be conceived to cause the origination of new races or species, or accelerate a differentiation already initiated (*cf.* DOBZHANSKY, 1951). Sublethality or lethality in the F_1 hybrids between two species may thus be conceived to imply that the origination of the species has been supported by their genic antagonism. It is, however, also conceivable that such an antagonism may be due to pure chance. Just as one or several mutations may by chance entail a hybrid incapacity between two lines of a species, inasmuch as the F_1 hybrids become inviable in consequence of complementary gene action, so also may the separation between two species by chance be further marked through a lethality barrier (or one comparable therewith) in consequence of mutative changes — positive or indifferent for the species itself, but fatal in combination with the genome of the other — in the one or both after the development of the

two species from the common origin has taken place. Of the various cases of lethality or sublethality between species or races through complementary genes it is only in connection with »corky» that there is an actual indication that the complementary genes actually served as an isolating mechanism, inasmuch as these genes are concentrated to regions where both the species occur (STEPHENS, 1946, 1950).

As regards the hybrids between *L. hybridum* and tetraploid *L. purpureum*, the two circumstances that the one component is an allotetraploid and that this is not cross compatible with diploid *L. purpureum* do not in themselves constitute any obstacle to a species differentiation within *Lamium* having once been promoted by the gene barrier now disclosed. This may have isolated *L. purpureum* from the one of *L. hybridum*'s two parent species. What does, on the other hand, prevent us from assuming that a potential barrier ever effectively contributed to a species differentiation is the fact that *L. purpureum* is in a high degree autogamic, and that circumstances favour rather the assumption that it was so also earlier in its development than that it developed from allogamy to autogamy (BERNSTRÖM, 1953 b). If, on the other hand, the complementary gene system with *L. hybridum* has been of any importance in the origin of *L. purpureum*, this may be conceived to have been the case only in the area common to the two separating species, or — and perhaps rather — at an early stage of *L. purpureum*'s existence its complementary gene disappeared from part of the species through mutation. The absence of other differentiation within the species than that constituted by the occurrence or lack of the complementary gene for dwarfish growth would seem to indicate this (*cf.* p. 16).

Naturally it is also possible to assume that the complementary gene system for dwarfish growth found has served as a species-differentiating agent at an earlier stage in the history of the genus. If this has not been the case there are nevertheless signs which may indicate that the complementary gene (probably one) in *L. purpureum* for dwarfish growth in the hybrids with *L. hybridum* has a similar though for the most part weaker effect also in other species hybrids. Hybrids between *L. intermedium* and tetraploid *L. purpureum* have thus proved able to become either weak, disharmonious plants or large and completely normal. Reasons have been adduced above which favour the assumption that the difference may be due to the genotype in the *L. purpureum* lines. The triploid hybrids between tetraploid *L. purpureum* and diploid *L. amplexicaule* were all normally developed, but after doubling of the chromosome number the hybrids in which *p7* entered proved consider-

ably weaker than those with *p*42, and were also characterized by various disharmonious features. One may as an explanation of this perhaps conceive that there was a slight negative complementary gene effect in the hybrids with *p*7, which was counteracted in the triploid hybrids by heterosis and perhaps also by the fact that the triploid chromosome number was the most favourable for the vegetative development, in the same way as, *e. g.*, triploid sugar beet and aspen have proved superior to the diploid plants (RASMUSSON, 1947, and unpubl.; JOHNSSON, 1951). In the hexaploids, on the other hand, the unfavourable gene effect could manifest itself. One must here, however, not forget that increase of the chromosome number far beyond its optimum has very different effects even on pure lines. Thus, octoploid plants of a line of *L. hybridum* turned out considerably poorer and more disharmoniously developed than of two others (BERNSTRÖM, unpubl.).

The crossability between tetraploid *L. purpureum* and diploid *L. amplexicaule* is not affected by the complementary gene for dwarfish growth in the former, as is seen from data published earlier (BERNSTRÖM, 1953 a, Table 3). *L. bifidum* subsp. *balcanicum*, on the other hand, was quite cross compatible with *p*53, but not at all with *p*29 (p. 28). It may be a coincidence, however, that it was the line of *L. purpureum* whose tetraploid produces normally developed hybrids with *L. hybridum* that was here the crossable one, while *p*29 was non-crossable and also entails dwarfish growth for the hybrids with *L. hybridum*; as will be shown below, the difference in crossability can scarcely depend on the complementary genes for dwarfish growth. Further cross experiments to throw light upon the question proved a failure owing to the refusal of *L. bifidum* subsp. *balcanicum* to flower chasmogamically. If, however, one tries to summarize the cases of dwarfish growth and incompatibility which have been manifested in the material studied in a simple explanation valid for all of them, it proves extremely difficult to allot to *L. bifidum* subsp. *balcanicum* the complementary gene or genes occurring in *L. hybridum*, and giving rise to dwarfish growth in the hybrids with certain of the tetraploid lines of *L. purpureum*. The difficulty resides in the fact that the hybrids between *L. purpureo-bifidum* and *p*61 are normally developed, whereas the hybrids of this line with *L. hybridum* become dwarfs; and conversely, the hybrids between *L. purpureo-bifidum* and *p*60 are slightly disharmonious, whereas those between *L. hybridum* and *p*60 are completely normal.

As cross pollinations have been made between *L. bifidum* subsp. *bal-*

canicum and but two lines of *L. purpureum*, one must for the present ascribe the incompatibility between the former and *p*29 to another cause than that discussed above, and proceed on the assumption that the line of *L. bifidum* subsp. *balcanicum* studied lacks the complementary gene (or genes) for dwarfish growth which occurs in *L. hybridum*. This by no means necessarily implies that *L. bifidum* subsp. *balcanicum* may not be the one parent of *L. hybridum*; some lines of *L. bifidum* subsp. *balcanicum* may have a complementary gene for dwarfish growth while others lack this, in the same way as is the case in *L. purpureum*.

If in the b_h genome in *L. hybridum* we assume the occurrence of a complementary gene, or group of genes, designated *XX*, which is assumed to have a growth-inhibiting effect with *zz* in *L. purpureum*, while with *ZZ* it has no injurious influence upon the vegetative development, and *Z* is incompletely dominant over *z*, this might be the simplest possible explanation, and at the same time one which in consideration of the limited scope of the material is rather satisfactory, of the dwarfish growth in the F_1 hybrids between the two species, and also of the inheritance of this in the hybrids of the type *L. hybridum*$\times F_1$ [*ZZZZ*$\times$*zzzz*]. *L. hybridum* would then have the constitution *XXZZ*, lines of the type *p*60 *ZZZZ* and of *p*61 *zzzz*. Hybrids with *XZZz* would be »dwarf-stamped», and those with *XZzz* would be dwarfs. Conceivable causes of the different segregation ratios in the hybrids *L. hybridum*$\times$ F_1 (*ZZZZ*$\times$*zzzz*) have been adduced above (p. 24).

The complementary gene system might be assumed to include, in the *a* genome, an allele to *X* called *x*, with a complementary effect reminiscent of that of *X*, though much weaker than this. (According to the above reasoning, the possible allele in the *b* genome analysed would have to be designated, *e. g.*, x_0.) This would explain the weak growth in the majority of the hybrids between *L. intermedium* and tetraploid *L. purpureum*, if the constitution of the former is assumed to be *xxZZ*; and it would explain the poor vitality of *L. purpureo-amplexicaule 1*, but be in bad accordance, on the other hand, with the normal growth in the corrresponding triploids. It must, however, be understood that in view of the limited scale of the experiments the attempt to explain the hereditary basis of dwarfish growth, *etc.*, within the genus must necessarily be tentative and cannot be carried out in any detail. The aim of the discussion is to point out the possibility that the complementary gene system may recur in several of the species. As a matter of fact there is no proof even that the complementary factor or factors

designated *XX* belong at all to the b_h genome; this assumption is supported only by the two facts that no intraspecific F_1 hybrids of *L. purpureum* have turned out dwarfs, and that this species must be assumed to be the one parent species to *L. hybridum*.

The slight disharmony characterizing the F_1 plants of *L. purpureo-bifidum* × *p*60 was an effect of over-representation of *p*60 and was probably accentuated by the tetraploidy. The hybrids between *p*53 and *L. bifidum* subsp. *balcanicum* were completely harmoniously developed, and *p*53 was the diploid line from which *p*60 originated. In this case there was thus no negative complementary gene effect of the kind discussed above.

As regards, finally, the more or less pronounced dwarfish growth in the F_1 and F_2 of *L. hybridum* × *L. purpureo-bifidum*, it has proved difficult to connect it reasonably with the complementary genes for dwarfish growth in the F_1 between *L. hybridum* and tetraploid *L. purpureum*. As far as is shown by the unfortunately small numbers of individuals of the various types in the F_2, however, the poorer plant growth and the dwarfish growth may conceivably be due to the complementary effect of two pairs of alleles, so that *AABB* and *aabb* represented the parents, while in F_2 plants that were heterozygous in respect of the one and homozygous in respect of the other of *A* and *B*, or were double heterozygous, had more or less the dwarf-stamp, and plants that were homozygous in respect of *a* or *b*, and homozygous or heterozygous for *A* or *B*, were dwarfs. The expected segregation ratio would thus be two normally developed : eight dwarf-stamped : six dwarfs, which is close to the numbers found, or 3 : 7 : 8. It is at present not possible to decide whether we have here to do with the effects of genes which are localized to the *L. purpureum* genomes (which *L. hybridum* is then assumed to possess), or to the two alternative genomes, or with genes which in the one parent or both of the parents are distributed between their two respective genomes. In intraspecific F_2 generations of diploid *L. purpureum* dwarf plants have actually segregated, though in proportions that did not agree with those found here (BERNSTRÖM, 1953 b).

ACKNOWLEDGEMENTS

For help with this and earlier published investigations I am indebted to many persons. My teacher, Professor A. MÜNTZING, who suggested the subject for my thesis, has followed my work with interest, and I am

warmly indebted to him for valuable advice and active support. I am also very grateful to my present chief, Professor J. RASMUSSON, for the opportunities he has afforded me of completing the investigation at the Hilleshög Institute and of dealing with the material by the side of my ordinary work. For placing greenhouse facilities at my disposal for experimental material for four years I am very thankful to Professor N. HERIBERT NILSSON, and for corresponding services for one year I wish to convey my thanks also to Director G. WEIBULL. For valuable advice and discussions in connection with the work I wish especially to thank Professor A. HÅKANSSON, Docent G. ÖSTERGREN, fil. lic. A. LUNDQVIST, and fil. kand. S. ELLERSTRÖM.

My wife, Mrs. BITTE BERNSTRÖM, has—together with other assistants—extended to me valuable technical aid during a succession of years. My brother, jur. kand. GUSTAF BERNSTRÖM, has given me splendid support in connection with the translation of several papers. The gardener and the caretaker, G. HAGMAN and Y. VELANDER, have done me great service in their masterly care of the plant material.

Finally, I wish to make deeply grateful acknowledgement for the perpetual interest and unflagging support with which my father, fil. dr. GUSTAF BERNSTÖM, has followed my work through the years.

The investigations have been facilitated by financial support from the funds of the Royal Physiographical Society of Lund, and from the Nilsson-Ehle fund.

SUMMARY

(1) The aim of the investigation has in the first place been to go into the relationships between *Lamium purpureum* ($2n=18$), *L. amplexicaule* ($2n=18$), *L. intermedium* ($2n=36$), and *L. hybridum* ($2n=36$), particularly with reference to the question as to whether either or both of the two latter constitute allotetraploids of the two first-mentioned species. The investigations have also included a line of *L. bifidum* subsp. *balcanicum* ($2n=18$). They have been chiefly concentrated on the chromosome pairing in F_1 hybrids between species as well as artificially produced autotetraploids and various synthetic allopolyploids, and on attempts to synthesize the tetraploid species. In addition to this, studies have been carried out on the hereditary causes of dwarfish growth in species hybrids, the properties of artificial allopolyploids, including newly formed lines of *L. intermedium*, and the

properties of the F_2 generations of especially *L. hybridum*×tetraploid *L. purpureum*.

(2) The chromosome pairing in the F_1 hybrids between tetraploid *L. purpureum* and diploid *L. amplexicaule* took place almost exclusively in 9 II+9 I. Occasional trivalents indicated slight partial homology between the genomes of the two species. The hybrids were practically sterile, and particularly as regards the form of the calyces were reminiscent of *L. intermedium*.

(3) Two plants in the F_2 after tetraploid *L. purpureum*×diploid *L. amplexicaule* were pure diploid *L. purpureum*, whose appearance must be due to the fact that all *amplexicaule* univalents in I A sometimes went to the one pole. Another plant was likewise, to judge from its appearance, pure *L. purpureum*, with $2n=29$. Cytologically, the arising of a ±triploid *L. purpureum* plant is conceivable, and as triploids cannot possibly be produced through crossing between tetraploids and diploids, the hybrid mother tissue, in combination with a better numerical relation between the chromosome numbers of the mother tissue, the endosperm and the embryo than after crossing tetraploid× diploid, is assumed to have rendered possible the occurrence of the triploid plant.

One plant with $2n=39$ was very reminiscent of *L. intermedium*; it is presumed to have arisen after spontaneous cross-fertilization of *L. intermedium*, but the variation of the progeny within the frame of *L. intermedium*'s characters showed that the mother plant must have arisen from two almost equivalent gametes, and that *L. intermedium* must thus be an allotetraploid between *L. purpureum* and *L. amplexicaule*.

(4) In another progeny after triploid hybrids between tetraploid *L. purpureum* and diploid *L. amplexicaule* was obtained, after self-fertilization, a tetraploid plant with good fertility and which in its essential characters agreed with *L. intermedium*. F_1 hybrids between its progeny and *L. intermedium* had good seed-setting, and $>90\,\%$ pollen fertility. The new line must be considered to belong to *L. intermedium*, and constitutes still another proof that the latter is an allotetraploid between *L. purpureum* and *L. amplexicaule*.

(5) The chromosome pairing in the F_1 hybrids between *L. intermedium* and autotetraploids of *L. purpureum* and *L. amplexicaule*, respectively, occurred predominantly in trivalents + univalents. Bivalents were common, and occasional quadrivalents occurred. Higher contents of these latter in the hybrids mentioned than of trivalents in the F_1 between tetraploid *L. purpureum* and diploid *L. amplexicaule*

indicate, in combination with the cytological and genetical homogeneity of *L. intermedium*, that chromosome-structural changes may have taken place in connection with the appearance of the latter.

(6) The chromosome pairing in the F_1 hybrids between *L. hybridum* and tetraploid *L. purpureum* was likewise characterized by a preponderant occurrence of trivalents+univalents, proving that *L. hybridum* must be an allotetraploid. Occasional quadrivalents occurred. The hybrids developed only five to ten seeds per plant. There were different degrees of as well pollen sterility as seed sterility between hybrids with different lines. Corresponding differences existed also in the F_2. Thus, despite a high degree of chromosomal sterility also genic differences proved able to exert an influence upon the degree of sterility. After doubling the chromosome number in the F_1 hybrids with colchicine treatment the seed-setting of the octoploids proved to be much better than that of the tetraploids, but was quickly worsened in subsequent generations, probably as a result of increasing chromosomal unbalance.

(7) The continuous variation in the chromosome numbers in the F_2 of *L. hybridum*×tetraploid *L. purpureum* extended from $2n=29$ to $2n=44$. The distribution was pronouncedly skew, the class with 35 chromosomes being the most represented one. Factors regulating the distribution of the chromosome numbers are discussed, and this is compared with that occurring in autotetraploids within *Lamium*. The seed-setting showed a slight positive correlation with the chromosome number, and the pollen fertility and chromosome number showed a significant positive correlation. Plant height and vigour showed a slightly negative correlation with the chromosome number. This may conceivably be a consequence of a harder selection of the gametes the lower their chromosome number is. Conversely, the improvement of the fertility with increased chromosome number is probably due to a proportional increase of gametes with approximately chromosomal and genic balance.

There was a significant positive correlation between size of flower and pollen fertility, even after statistical elimination of the effect of vigour of plant and chromosome number. The correlation may be conceived to be caused either by linkage between factors causing sterility and factors affecting size of flower, or by a physiological influence on size of flower exercised by the degree of sterility. Examples are given of apparently similar correlations in other forms of the experimental material which rather favour the latter alternative.

(8) Diploid hybrids between *L. purpureum* and *L. bifidum* subsp.

balcanicum are completely sterile and summer annuals like the former, but in contradistinction to the latter. After doubling of the chromosome number the allotetraploid became rather fertile and practically constant. The chromosome pairing in bivalents was very regular, and only very few trivalents occurred. *L. purpureo-bifidum* was in appearance most reminiscent of *L. hybridum*, but diverged in several respects.

The F_1 hybrids between *L. hybridum* and *L. purpureo-bifidum* were characterized by a slight reduction of the pollen fertility. In about one third of the PMC's there occurred two to four univalents. The hybrids were more weakly developed than the parents, and in the F_2 dwarfish plants occurred.

(9) For both cytological and systematic reasons, which are discussed in detail, *L. hybridum* is assumed to be most probably an allotetraploid between *L. purpureum* and *L. bifidum* or its subsp: *balcanicum*. The grounds for the assumption of *L. hybridum*'s origination once as against several times are discussed. The conclusion primarily arrived at is that *L. hybridum* originated only once a long time ago, and that varieties of the presumed parent species entered, which as concerns either or both of them have been genotypically rather unlike those constituting *L. purpureo-bifidum*.

(10) F_1 hybrids between *L. hybridum* and tetraploid *L. purpureum* have either a fully normal development or develop as dwarfs. All the examined lines of *L. hybridum* carry one or several complementary genes for dwarfish growth, which co-operate with (probably) only one gene in *L. purpureum*. This occurred in 8 of 14 analysed lines, and has not been found to be connected with any genetic or geographic division of the species. From unknown causes the segregation ratios were significantly different after the crossing of two different lines of *L. hybridum* with the same interlinear F_1 hybrids of tetraploid *L. purpureum* of the type (»dwarf»×»normal»).

Reasons are adduced which rather contradict than support the assumption that the gene in *L. purpureum* for dwarfish growth in hybrids with *L. hybridum* played a rôle as species-differentiating agent in the earlier history of *L. purpureum*. The possibilities of connecting weakened plant development or incompatibility in other species hybrids within *Lamium*, which are described, with the same complementary genes as cause dwarfish growth in *L. hybridum*×tetraploid *L. purpureum* are discussed.

(11) The chromosome pairing, the cytological instability and the effect of aneuploid chromosome numbers upon different properties in

allohexaploid hybrids between *L. hybridum* and *L. amplexicaule* have been studied. In I M there occurred predominantly bivalents, 0—4 multivalents and 0—8 univalents. This confirms the observation that the homology, shown in the triploid hybrids, between one of the genomes from *L. hybridum* and the *L. amplexicaule* genome is deficient. The chromosome numbers in the hexaploids showed a general tendency to reduction. The seed-setting, which in autotetraploids of *Lamium* is the most sensitive indicator of the degree of aneuploidy, was reduced far more in these with decreasing chromosome number than in hexaploid *L. hybrido-amplexicaule*. The general occurrence of, and tolerance towards, reduction of the chromosome numbers in the latter are in the first place ascribed to the hexaploid chromosome number, and to the circumstance that especially the *L. amplexicaule* genomes and the genomes in *L. hybridum* presumably deriving from *L. bifidum* probably represent extensive gene duplications.

(12) The chromosome pairing in the hybrids *L. hybrido-amplexicaule* $\times$ *L. intermedium* and *L. hybrido-amplexicaule* $\times$ tetraploid *L. amplexicaule* has been studied. The results agreed with what might have been expected in the light of observations from other species hybrids.

(13) A preliminary investigation has been carried out of the chromosome pairing in allohexaploids with four genomes of *L. purpureum* and two genomes of *L. amplexicaule*. The multivalent formation among the *L. purpureum* chromosomes appeared to be less than in autotetraploid *L. purpureum*. The seed fertility in the allohexaploids was very inconsiderable, and the plants were coarse and unevenly developed.

(14) Good seed-setting in an allotetraploid in relation to that of its autotetraploid parents, as may be presumed to be the case as regards *L. purpureo-bifidum*, is explained not as a consequence of regular bivalent formation alone, but of this in combination with a heterosis-like effect stimulating the fertility (p. 33).

(15) A survey is given of the observations made concerning crossability between species, new autopolyploids and synthesized allotetraploids and allohexaploids. The crossability has proved to be strictly regulated by quantitative and qualitative factors, so that, *e. g.*, no constellation of genomes has proved to be realizable in more than one way, despite several different attempts. The impossibility of assessing the relationship between species, *etc.*, with reference to the crossability between them is stressed. The possibility for *L. intermedium* to arise despite the impossibility of hybridizing the diploid parent species is discussed.

Literature cited

BERNSTRÖM, P. 1949. Cytogenetic studies in the genus *Lamium*. — Proc. 8th Int. Congr. Genetics (Hereditas, Suppl. Vol.).

— 1950 a. Does the fertility of intraspecific hybrids in autogamous species improve in consequence of a heterotic effect? — Hereditas XXXVI.

— 1950 b. Cleisto- and chasmogamic seed setting in di- and tetraploid *Lamium amplexicaule*. — Hereditas XXXVI.

— 1952. Cytogenetic intraspecific studies in *Lamium*. I. Hereditas XXXVIII.

— 1953 a. Increased crossability in *Lamium* after chromosome doubling. — Hereditas XXXIX (Festskrift för ARNE MÜNTZING 2.3.1953).

— 1953 b. Cytogenetic intraspecific studies in *Lamium*. II. — Hereditas XXXIX.

— 1954. Fertility and aneuploidy in new autotetraploids in *Lamium*. — Hereditas XL.

BRINK, R. A. and COOPER, D. C. 1947. The endosperm in seed development. — Bot. Review 13.

BUCHHOLZ, J. T. and BLAKESLEE, A. F. 1930. Pollen-tube growth of the primary mutant of *Datura*, Rolled, and its two secondaries. — Proc. Nat. Acad. Sci. 16.

CHUKSANOVA, N. 1939. Karyotypes of pollen grains in triploid *Crepis capillaris*. — Comp. Rend. Acad. Sci. U. S. S. R. 25.

CLAUSEN, J., KECK, D. and HIESEY, W. 1945. Experimental studies on the nature of species. II. Plant evolution through amphiploidy and autoploidy, with examples from the *Madiinae*. — Carnegie Inst. Washington Publ. 564.

CLAUSEN, R. E. 1941. Polyploidy in *Nicotiana*. — Amer. Naturalist 75.

DARLINGTON, C. D. 1937. Recent advances in cytology. — London.

DARLINGTON, C. D. and LA COUR, L. F. 1942. The handling of chromosomes. — London.

DARLINGTON, C. D. and MATHER, K. 1949. The elements of genetics. — London.

DOBZHANSKY, TH. 1951. Genetics and the origin of species. — New York (3rd edition).

EAST, E. M. 1935. Genetic reactions in *Nicotiana*. I. Compatibility. — Genetics 20.

— 1936. Heterosis. — Genetics 21.

EINSET, J. 1947. Aneuploidy in relation to partial sterility in autotetraploid lettuce (*Lactuca sativa* L.). — Amer. Journ. Bot. 34.

FIORI, A. 1925—29. Nuova flora analitica d'Italia. Vol. II. — Florence.

GAJEWSKI, W. 1953. A fertile amphipolyploid hybrid of *Geum rivale* with *G. macrophyllum*. — Acta Soc. Bot. Poloniae 22.

GERSTEL, D. U. 1954. A new lethal combination in interspecific cotton hybrids. — Genetics 39.

GOODSPEED, T. H. 1945. Cytotaxonomy of *Nicotiana*. — Bot. Review 11.

GRANT, U. 1952. Cytogenetics in the hybrid *Gilia millefoliata*×*achilleaefolia*. I. Variations in meiosis and polyploidy rate as affected by nutritional and genetic conditions. — Chromosoma 5.

GUSTAFSSON, Å. 1951. Mutations, environment and evolution. — Cold Spring Harbor Symp. Quant. Biol. 16.

HAGBERG, A. 1953. Further studies on and discussion of the heterosis phenomenon. — Hereditas XXXIX.

HALL, O. L. 1954. Hybridization of wheat and rye after embryo transplantation. — Hereditas XL.

HAYEK, A. and MARKGRAF, F. 1931. Prodromus florae peninsulae Balcanicae. Vol. 2. — Rep. spec. nov. regni veg., ed. F. FEDDE. Vol. 30, 2.

HIORTH, G. 1942. Zur Genetik und Systematik der *amoena*-Gruppe der Gattung *Godetia*. — Zschr. ind. Abst.- u. Vererbl. 80.

HOLLINGSHEAD, L. 1930. A lethal factor in *Crepis* effective only in an interspecific hybrid. — Genetics 15.

HUTCHINSON, J. B. 1932. The genetics of cotton. Part VII. »Crumpled»: A new dominant in Asiatic cottons produced by complementary factors. — Journ. Genetics 25.

JOHNSSON, H. 1951. Lövträd. Asp och poppel. — Svensk växtförädling. Del II. Stockholm.

JÖRGENSEN, C. A. 1927. Cytological and experimental studies in the genus *Lamium*. — Hereditas IX.

KOSTOFF, D. 1941—43. Cytogenetics in the genus *Nicotiana*. — Sofia.

LAMM, R. 1945. Cytogenetic studies in *Solanum* sect. *Tuberarium*. — Hereditas XXXI.

LEVAN, A. 1936. Zytologische Studien on *Allium Schoenoprasum*. — Hereditas XXII.

— 1942. Studies on the meiotic mechanism of haploid rye. — Hereditas XXVIII.

— 1945. A haploid sugar beet after colchicine treatment. — Hereditas XXXI.

LITTLE, TH. M. 1945. Gene segregation in autotetraploids. — Bot. Review 11.

MCCLINTOCK, B. 1929. A cytological and genetical study of haploid maize. — Genetics 14.

MELCHERS, G. 1939. Genetik und Evolution. — Ztschr. ind. Abst.- u. Vererbl. 76.

MÜNTZING, A. 1926. Ein Art-Bastard in der Gattung *Lamium*. — Hereditas VII.

— 1930. Outlines to a genetic monograph of the genus *Galeopsis*. — Hereditas XIII.

— 1933. Hybrid incompatibility and the origin of polyploidy. — Hereditas XVIII.

— 1937. The effects of chromosomal variation in *Dactylis*. — Hereditas XXIII.

— 1939. Studies on the properties and the ways of production of rye-wheat amphidiploids. — Hereditas XXV.

MYERS, W. M. 1944. Cytological studies of a triploid perennial ryegrass and its progeny. — J. Heredity 35.

NORDENSKIÖLD, H. 1945. Cytogenetic studies in the genus *Phleum*. — Acta Agricult. Suecana I.

O'MARA, J. G. 1950. The effects of chromosome substitution on competition between gametes. — Genetics 35 (Abstr.).

PETO, F. H. 1933. The cytology of certain intergeneric hybrids between *Festuca* and *Lolium*. — Journ. Genetics 28.

RANDOLPH, L. F. and MCCLINTOCK, B. 1926. Polyploidy in *Zea mays* L. — Amer. Naturalist 60.

RASMUSSON, J. 1947. Olika kromosomtals inverkan på sockerbetornas egenskaper. — Nord. Jordbruksforskares För:s Kongressberetn., 2, Del A.

SANSOME, F. W. and PHILP, J. 1939. Recent advances in plant genetics. — London (2nd edition).

SAX, K. 1933. Species hybrids in *Platanus* and *Campsis*. — Journ. Arnold Arboretum 14.

SCHLÖSSER, L. A. 1936. Befruchtungsschwierigkeiten bei Autopolyploiden und ihre Überwindung. — Der Züchter 8.

SEARS, E. R. 1941. Chromosome pairing and fertility in hybrids and amphidiploids in the *Triticinae*. — Missouri Agr. Exper. Station, No. 337.

SEARS, E. R. 1944. Inviability of intergeneric hybrids involving *Triticum monococcum* and *L. aegilopoides*. — Genetics 29.

SELANDER, S. och BRYANT-MEISNER, R. 1909. Blombesökande insekter på Kullen 1908. — Sv. Bot. Tidskr. 3.

SILOW, R. A. 1941. The comparative genetics of *Gossypium anomalum* and the cultivated Asiatic cottons. — Journ. Genetics 42.

SMITH, H. H. 1952. Relation between sterility and morphological characters in an interspecific *Nicotiana* cross. — Genetics 37.

STEBBINS, G. L. JR. 1945. The cytological analysis of species hybrids. II. — Bot. Review 11.

— 1949. The evolutionary significance of natural and artificial polyploids in the family *Graminae*. — Proc. 8th Intern. Congress Genetics (Hereditas, Suppl. Vol.).

— 1950. Variation and evolution in plants. — New York.

STEPHENS, S. G. 1946. The genetics of 'corky'. I. The New World alleles and their possible role as an interspecific isolating mechanism. — Journ. Genetics 47.

— 1950. The genetics of 'corky'. II. Further studies on its genetic basis in relation to the general problem of interspecific isolating mechanisms. — Journ. Genetics 50.

STOYANOFF, N. and STEFANOFF, B. 1925. Flore de la Bulgarie. Vol. 2. — Sophia.

UPCOTT, M. B. 1935. The cytology of triploid and tetraploid *Lycopersicum esculentum*. — Journ. Genetics 31.

VELENOVSKY, J. 1893. Dritter Nachtrag zur Flora von Bulgarien. — Ber. Böhm. Ges. Wiss. 1893, No. 37.

WATKINS, A. 1932. Hybrid sterility and incompatibility. — Journ. Genetics 25.

WICKBOM, T. 1945. Cytological studies on *Dipnoi*, *Urodela*, *Anura* and *Emys*. — Hereditas XXXI.

WIEBE, G. A. 1934. Complementary factors in barley giving a lethal progeny. — Journ. of Heredity 25.

WINKLER, H. 1916. Über die experimentelle Erzeugung von Pflanzen mit abweichenden Chromosomenzahlen. — Ztschr. f. Bot. 8.

Contents

Introduction 1
Material and methods 2
Experimental 3

I. Tetraploid F_1 hybrids 3

A. *L. intermedium*×tetraploid *L. purpureum* 3

a. Cytology 3
b. Fertility and morphology 6

B. *L. intermedium*×tetraploid *L. amplexicaule* 8

a. Cytology 8
b. Fertility and morphology 10

C. *L. hybridum*×tetraploid *L. purpureum* 12
1. Tetraploid hybrids 12
a. Cytology 12
b. Morphology, and genetics of hybrid dwarfishness 14
c. Fertility 25
2. Octoploid hybrids 27

II. Diploid and triploid F_1 hybrids, their corresponding allopolyploids, and hybrids with them 28

A. *L. purpureum*×*L. bifidum* subsp. *balcanicum* 28
1. The diploid hybrids 28
2. Allotetraploid *L. purpureo-bifidum* 29
a. Cytology 29
b. Fertility and morphology 31
3. *L. hybridum*×*L. purpureo-bifidum* 36
a. Cytology 36
b. Fertility and morphology 36
4. *L. intermedium*×*L. purpureo-bifidum* 38
5. *L. purpureo-bifidum*×tetraploid *L. purpureum* 39

B. *L. hybridum*×*L. amplexicaule* 40
1. The triploid hybrids 40
2. Allohexaploid *L. hybrido-amplexicaule* 41
a. Cytology 41
b. Aneuploidy, fertility and morphology 44
3. *L. hybrido-amplexicaule*×*L. intermedium* 50
a. Cytology 50
b. Fertility and morphology 52
4. *L. hybrido-amplexicaule*×tetraploid *L. amplexicaule* 53
a. Cytology 53
b. Fertility and morphology 54

C. Tetraploid *L. purpureum*×*L. amplexicaule* 55
1. The triploid hybrids 55
a. Cytology 55
b. Fertility and morphology 57
2. Allohexaploid *L. purpureo-amplexicaule* 60
a. Cytology 60
b. Fertility and morphology 62
3. *L. purpureo-amplexicaule*×*L. hybrido-amplexicaule* 63

III. Investigations on some F_2 and subsequent generations from interspecific hybrids 63

A. Offspring from hybrids between tetraploid *L. purpureum* and diploid *L. amplexicaule* 63
1. First origination of new *L. intermedium* 63
a. The original plant and its sister plants 63
b. Subsequent generations of new *L. intermedium* 68
2. Second origination of new *L. intermedium* 74

B. Investigations on F_2 generations from *L. hybridum*×tetraploid *L. purpureum* 78
1. F_2 from hybrids of normal growth 78
a. Morphology and fertility 78
b. Chromosome number variation 82
c. Effects of the numerical chromosome variation 85
2. F_2 from dwarfish hybrids 91

C. Investigations on F_2 generations from *L. intermedium*×tetraploid *L. amplexicaule* 93

Discussion 94
I. Cross compatibility and incompatibility 94
II. Relationships between the species primarily as indicated by degree of homology between their genomes 98
III. Hybrid dwarfishness within *Lamium* 107

Acknowledgments 112
Summary 113
Literature cited 118

FACTORS INFLUENCING RADIATION INDUCED LETHALITY, STERILITY, AND MUTATIONS IN BARLEY

By *LARS EHRENBERG*
INSTITUTE OF ORGANIC CHEMISTRY AND BIOCHEMISTRY, UNIVERSITY OF STOCKHOLM

IN laboratory experiments in which the inhibition of the growth rate of barley seedlings was measured after irradiation of seeds, the radiation induced injury was found to decrease with increasing water content of the seeds, within the range of 7—20 per cent water; to be greater at a lower germination temperature, and to depend on the storage conditions in the interval between irradiation and sowing (EHRENBERG, 1955 a). The influence of these factors was greater in the case of radiations with a low ion density (linear energy transfer) — *e. g.*, γ-rays and X-rays — than when more densely ionizing radiations, *e. g.*, fast neutrons, were used. The influence was of such magnitude that the factors studied sufficed to explain the variations generally obtained in and between radiation experiments.

With the above-mentioned experience as the basis of approach the present investigation was concerned with a study of the influence of the moisture of the seeds, at the moment of irradiation, on the lethality, sterility, and mutation rate increase produced by radiations. At the same time, mitotic disturbances in seedling roots were also investigated. Further, special arrangements made it possible to study the influence of temperature during germination and seedling growth, and to some extent, of storage before sowing, on the radiation effects.

EXPERIMENTAL

Material and General Experimental Plan. — The material consisted, as in the earlier laboratory experiments (EHRENBERG, 1955 a), of a sifted sample of ripe kernels of the commercial two-rowed barley variety, Bonus.

Two experiments will be reported here, *viz.*:

(1) In the spring of 1953, samples of the same origin were equilibrated simultaneously at Lund and Stockholm with air of different

relative humidities and irradiated, respectively, with X-rays (at the Institute of Genetics, Lund) and fast neutrons (at the Nobel Institute for Physics, Stockholm). For the purpose of studying the influence of storage on the radiation effects, the X-irradiated samples were divided into two parts, which were sown with an interval of about one week. (Due to technical difficulties in delivering identical neutron doses to sufficiently large samples, this subdivision had to be restricted to the X-irradiated material.) The X-ray series yielded peculiar results, which probably had to be viewed in the light of a period of cold weather influencing the material sown first, rather than as an influence of the storage.

(2) In the following spring a preliminary experiment was therefore conducted, seeds being sown, immediately after irradiation, in forcing-beds, one of which was covered and heated, the other not, but which were otherwise identical. In order to ensure adequate tillering (*i. e.*, a sufficient number of spikes), the coverings had to be removed at a seedling height of about 15 cm. In this connection it must be pointed out that due to the great difficulty of obtaining normal mature plants in the greenhouse, where temperature, humidity, and light could be varied, studies of this kind have to be conducted as field experiments, taking the conditions as they are. When the present experiments were planned, it was not known that the temperature influence, at least as regards the growth inhibition in seedlings, seems to be restricted to an early stage of germination. Whether this also applies to the effects observed at the stage of maturity or in the following generation, will be investigated in later experiments.

Seed Moisture. — The water content of the material to be X-irradiated was changed by pumping air of 0, 30, and 100 per cent humidity, for 9 days at 23° C. and at a rate of 100 ml per min, through samples of 10,000 seeds (*i. e.*, about 500 cm^3), which were placed in 500 ml measuring glasses. Zero per cent humidity was obtained by passing the air through one wash-bottle with concentrated H_2SO_4 followed by a U-tube with silica gel; in order to obtain 30 and 100 per cent humidity, the air was passed, respectively, through two wash-bottles with 12,5 M KOH, and three wash-bottles with water. Since CO_2 is absorbed in the alkali used to obtain 30 per cent humidity, CO_2 free air was used, for the sake of conformity, at the other humidities, too. Immediately before irradiation, the water contents of the seeds were determined by drying at 105° C., and the following values were obtained:

Humidity of air	Water content of seeds
0 per cent	$8{,}6 \pm 1{,}3$ per cent
30 » »	$10{,}5 \pm 0{,}9$ » »
100 » »	$18{,}1 \pm 0{,}03$ » »
untreated (*i. e.* sample stored in contact with room air)	12,5 » »

The limits of variation given are the differences between air entrance (bottoms of the measuring glasses) and exit (tops of glasses). With the procedure employed the water content was obviously changed more effectively at the air entrance, where the lower limits of the values for 0 and 30 per cent were obtained.

The material to be irradiated with neutrons was treated in a similar way, at a somewhat lower temperature (20° C.). Since in this case smaller samples were used, full homogeneity as to the change of the water content within the samples was obtained:

Humidity of air	Water content of seeds
0 per cent	8,4 per cent
30 » »	11,5 » »
100 » »	21,6 » »
untreated	11,0 » »

In the irradiations, »untreated» samples were also included.

Irradiations. — After homogenization each sample was subdivided into portions to be irradiated. In order to secure comparable mutation data uninfluenced by lethality, sterility, and such elimination phenomena (*cf.* EHRENBERG *et al.*, 1952, 1953) as are characteristic of the effects of high doses, low X-ray doses in the range of 300—1,000 r had to be applied, too. Since the mutation rate averages 10^{-5} mutations per r and per spike progeny (EHRENBERG *et al.*, 1953), and since about six spikes per plant can be investigated, 1,000 mature plants are required, at the lower dose limit, to yield about 15 mutation cases — a number which satisfactorily characterizes the mutation rate. Samples of 500—2,000 seeds were therefore irradiated with doses within the range of 313—15,000 r, the highest numbers of seeds at low and high doses (the latter in order to compensate for the decreased plant number due to lethality).

The X-ray machine at the Institute of Genetics, Lund, was run at 175 kV, without filtration, the seeds being placed in one layer in closed flat paper boxes. Dosimetry (and control of field homogeneity at the irradiation site) was done with a Victoreen type (Moxnes) bakelite-walled ionization chamber.

The fast neutrons were obtained by Cu(dn)Zn and other processes occurring when a copper target was bombarded with deuterons accelerated to 25 MeV in the 225 cm cyclotron at the Nobel Institute for Physics, Stockholm. Doses within the range of 150—1,000 rep (1 rep=93 erg g^{-1}) were applied, estimated from the oxidation of Fe^{2+} in sulfuric acid solution (EHRENBERG and SAELAND, 1954 a; EHRENBERG, 1955 b). Since the neutrons are 10—50 times more effective than X-rays in producing the effects studied (EHRENBERG and NYBOM, 1954), these doses are fairly high, but due to difficulties of measuring lower doses, the latter were not applied.

Field Treatment. — After taking a few seeds for special tests (*e. g.*, determination of chromosome aberrations; *vide* below), each irradiated sample was divided into two parts (*a* and *b*). All seeds were sown in the field at Uppåkra, near Lund. Of the neutron irradiated seeds both replications, *a* and *b*, were sown on the day (April 30) after irradiation. Of the X-irradiated seeds replication *a* was sown with low doses on May 1 and 2, and on May 3 and 4 (the two highest doses), whereas replication *b* was stored in paper bags at 23° until sowing on May 8—10. The individual kernels were sown at intervals of 5 cm in rows, the distance between which was 15 cm. Data for maximum and minimum temperatures as welll as precipitation, at Lund.

were obtained from the Swedish Meteorological and Hydrological Institute, Stockholm, for the period of germination and initial growth (Fig. 8).

Analyses. — The following properties of the material were determined:

Seed germination: the number of seedlings obtained at a control mean height of about 10 cm, *i. e.*, the counting was done about 3 weeks after sowing.

Plant survival: the number of mature plants at harvest.

(Mean) *Spike fertility*: the average frequency, per spike, of spikelets giving rise to seeds. From the fertility, F, the *radiation induced sterility*, S, was determined as 100 $(F_C - F_{irr}) / F_C$, F_C and F_{irr} being the fertilities of control and irradiated sample, respectively, and all quantities being expressed in per cent. Except in cases of too high a lethality (< 50 surviving plants) the fertility was determined from about 300 spikes.

The *mutation rate* was estimated according to GUSTAFSSON's methods (1940, 1947; *cf.* NYBOM, 1954) from the frequency of spikes giving rise, in the following generation, to seedlings with abnormal colour (due to disturbances in plastids and/or plastid borne pigments). All spikes from each plant (except one whose seeds were sown in the field for the study of vital mutants) were laid, unthreshed, in moist sand in the greenhouse, and the mutations were counted and classified at about 15 cm seedling height, 2—3 weeks after sowing [*cf.* NYBOM's (*l. c.*) figures 7 and 8]. In the self-fertilizer, barley, each spike in whose primordium a mutation has been induced will produce about 25 per cent seedlings (or less) homozygous for the mutated allele. Only rarely does a mutation extend to more than one spike of the plant. Hence the spikes, and not the plants, have been regarded as units in the calculation of mutation frequencies and fertilities.

The frequency of *chromosomal aberrations*: per cent of the first mitoses, in seedling roots, with fragments or bridges, was determined by Dr. O. GELIN, Landskrona. Some of the irradiated seeds were sown in Petri dishes at 20° C., and the roots were fixed at about 1 cm length.

The tables of BONNIER and TEDIN (1940) were used for estimations of significance.

The exploratory series irradiated and sown in forcing-beds in 1954 comprised seeds equilibrated with dry (0 %) and moist (100 %) air as well as seeds soaked for 24 hours at 20° C. in water. Each sample comprised only 50 seeds, only about half of which survived. Hence the only property determined was the sterility induced. In the heated part of the forcing-bed the temperature, as determined with a thermograph, was maintained at 10—20° C. (for the first 3 weeks of development of the plants), while the temperature in the unheated part varied between 0 and + 10° C.

RESULTS

The data from the field experiment of 1953 are summarized in Figs. 1—7. The results of the temperature experiment performed in 1954 are treated in the discussion (p. 139, Table 7).

The *mean spike fertilities* were found, in the X-irradiated series, to depend on the water content of the seeds at the moment of irradiation, the induced sterilities (S, *vide* above) decreasing in the order 0 % >

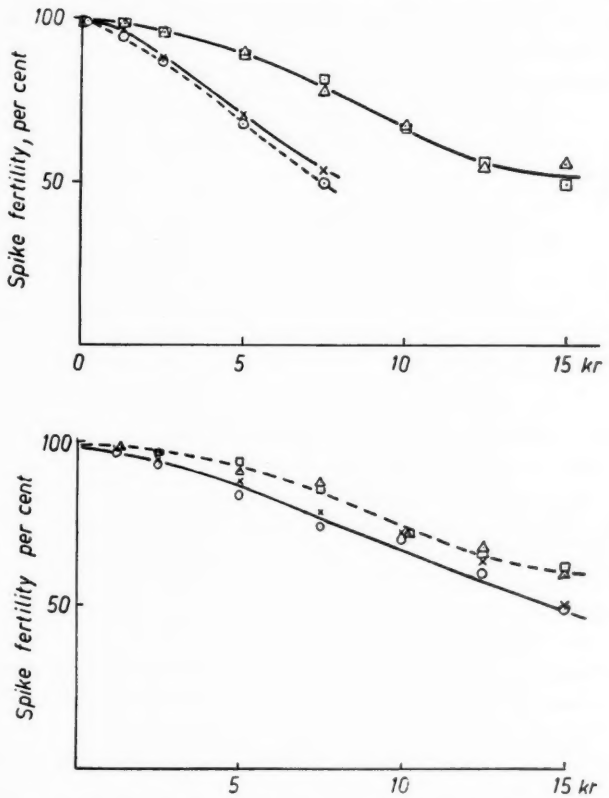


Fig. 1. Mean spike fertilities at different X-ray doses. Upper diagram: 0 % series, replication *a*: ×, *b*:⊙; and untreated series, replication *a*: △, and *b*:⊡. — Lower diagram: 30 % series, replication *a*: ×, and *b*⊙; and 100 % series *a*: △, and *b*:⊡.

$>$ 30 % $\sim$ untreated $>$ 100 % (*cf.* Fig. 1). The influence of the sowing time is less conspicuous, S_a (*i. e.*, sterility in replication *a*) being consistently less than S_b in the 0 and 30 % series, whereas at the higher water contents the points are distributed at random. In the neutron-irradiated material no influence of the water content of the seeds, at the moment of irradiation, on the sterility induced can be traced (Fig. 2). In the X-ray series the induced sterilities are exponential functions of the dose, with an elimination of the injury becoming evident at high doses, whereas the curve in the neutron series might well be

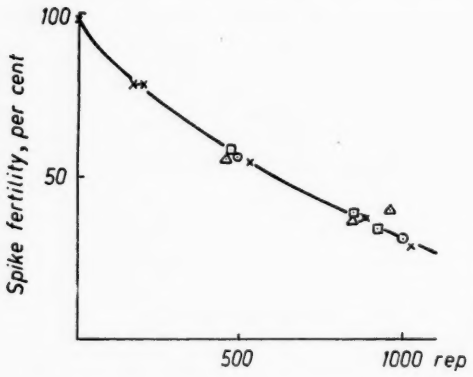


Fig. 2. Mean spike fertilities at different ueutron doses; 0 %: ⊙, 30 %: △, 100 %: ⊡ and untreated series: ×. The double-point at 180 rep shows the limits of error of the dose determination at this low dose.

rectilinear, at least at lower doses, any deviation being due to errors in the dosimetry; *cf.* the sterilizing effect of the relatively better defined doses of pile neutrons (EHRENBERG and SAELAND, 1954 b: Fig. 11).

In the present investigation we were primarily concerned with measuring in what degree the radiation required to produce a given effect is changed by the variation of such factors as the seed moisture at irradiation, or the sowing time, and with comparing the effectiveness changes for different radiation effects. In those cases where the effectiveness was changed by a certain factor independently of the dose, the difference between the logarithms of the numerical values for effects to be compared was determined, *e. g.*, $d = \log S_1 - \log S_2$, S_1 and S_2 being two sterilities compared, and the statistical significance of the average difference, $\bar{d} = \frac{d}{n}$ (n=number of comparisons), was determined. In the calculation of the standard error, m, of $\bar{d}$ it was not necessary to weigh the individual log values to their accuracies, since the relative standard errors of sterilities ($m_S \sim m_F$), and also of mutation rates, were about constant. The standard error, m, therefore, was simply determined as $\sqrt{\frac{(d-\bar{d})^2}{n(n-1)}}$, *i. e.*, the individual d values obtained at different doses and, in the comparison between seed moistures, in different replications, were regarded as measures of the effectiveness change caused by the factor varied, irrespective of the number of individuals upon which one d value was based. This treatment will not lead to overestimation of the significance. It is independent of both the dose function of the property investigated and the units used to express the effect.

TABLE 1. *Differences, $\bar{d}$, between logarithms of induced sterilities, with standard errors (m), variate numbers (n), t-values, and probabilities (p; cf.* BONNIER *and* TEDIN, *1940: Table II). In comparisons involving the 0 % series, the lowest dose, and involving the other series, the two lowest doses were excluded.*

Comparison	$\bar{d}$ (see text)	m	n	t	p
a—b, 0 % series	—0,077	0,014	5	5,5	0,001—0,01
» , 30 » »	—0,082	0,027	7	3,0	≈ 0,02
» , 100 » »	0,007	0,043	7	0,16	0,8—0,9
» , untreated series	—0,031	0,024	7	1,3	0,2—0,3
» , 30 % + untreated	—0,057	0,020	14	2,8	0,01—0,02
» , 0 % + 30 % + untreated	—0,062	0,019	19	3,3	0,001—0,01
» , all treatments	—0,044	0,018	26	2,4	≈ 0,02
0 %—30 %	0,366	0,023	8	16	≈ 0
30 » —untreated	0,045	0,025	14	1,8	0,05—0,1
30 » —100 %	0,172	0,037	14	4,6	$\approx 10^{-4}$

The analysis, according to this method, of the sterility data is summarized in Table 1. A fairly high degree of significance is indicated for $S_a < S_b$ at the two lowest water contents (0 and 30 %), whereas this

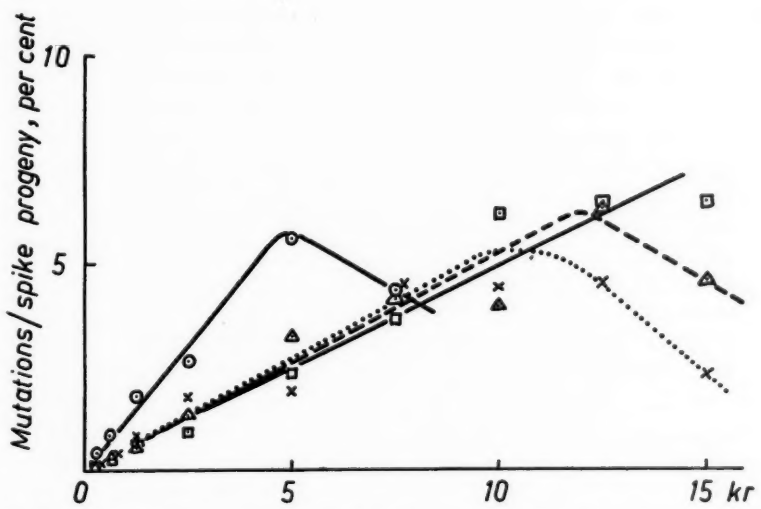


Fig. 3. Mutation rates at different X-ray doses. Mean values of replications a and b; 0 %: ⊙, 30 %: △, 100 %: ⊡, and untreated series: ×.

TABLE 2. *Mutation rates in the region of linear dose dependence (averages of values obtained at different doses).*

Radiation	Seeds treated with air of humidity %	Mutation rate per r and per spike progeny, ± standard error	
		a series	*b* series
X-rays	0	$(1{,}16 \pm 0{,}08) \cdot 10^{-5}$	$(1{,}34 \pm 0{,}12) \cdot 10^{-5}$
	30	$(0{,}53 \pm 0{,}04) \cdot 10^{-5}$	$(0{,}51 \pm 0{,}03) \cdot 10^{-5}$
	100	$(0{,}53 \pm 0{,}05) \cdot 10^{-5}$	$(0{,}45 \pm 0{,}02) \cdot 10^{-5}$
	untreated	$(0{,}60 \pm 0{,}06) \cdot 10^{-5}$	$(0{,}47 \pm 0{,}05) \cdot 10^{-5}$
neutrons	all humidities	$\sim 20 \cdot 10^{-5}$	

seems less probable for the two higher water contents. (Logically, it might be assumed that the untreated series occupies a position between 30 and 100 %, and closer to the former.) A high degree of significance is demonstrated for the differences between the 0 and 30 % series and between the 30 and 100 % series, the differences corresponding, on the linear scale, to effectiveness reductions amounting to 2,3 and 1,5 times, respectively. The 30 % and the untreated series are not significantly different.

The *mutation rates* exhibit, in the X-ray series, from low to medium doses a linear dependence on dose (*cf.* Fig. 3). Since the spontaneous mutation frequency, in barley varieties corresponding to the rate induced by 40—100 r (GUSTAFSSON, 1947), is too low to be detected, the curves start at the origin. At higher doses a conspicuous decrease of the mutation rates is observed, in accordance with several earlier experiments (*cf.* EHRENBERG *et al.*, 1952). The relative mutation rates in the

TABLE 3. *Differences, $\bar{d}$, between logarithms of mutation rates, with standard errors (m), variate numbers (n), t-values, and probabilities (p). The comparisons between water contents restricted to the rectilinear part of the curve.*

Comparison	$\bar{d}$ (see text)	m	n	t	p
a—*b*, 0 % series	—0,082	0,042	6	1,95	0,1—0,2
» , 30 % series	0,012	0,038	9	0,32	0,7—0,8
» , 100 % series	0,055	0,059	9	0,93	0,3—0,4
	(0,111) [1]	(0,026)	(8)	(4,3)	(0,001—0,01)
» , untreated series	0,101	0,033	9	3,1	0,01—0,02
0 % — 30 %	0,369	0,041	10	9	~ 0
30 % — untreated	—0,004	0,039	16	0,10	0,9—1
30 % — 100 %	0,023	0,036	16	0,64	0,5—0,6

[1] Calculation with one very deviating value, at 2,500 r, eliminated.

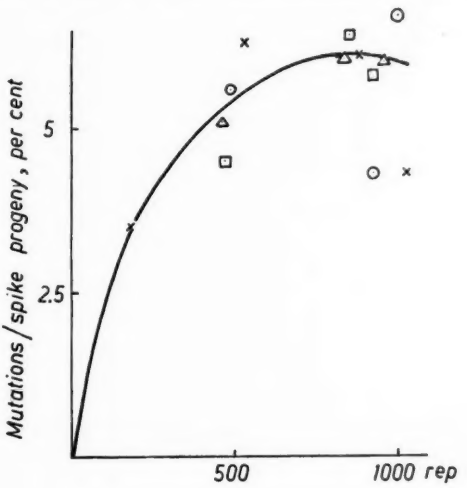


Fig. 4. Mutation rates in neutron series. Symbols as in Fig. 2. Abscissa: dose.

region of linear dose dependence are shown in Table 2. The mutation rate is highest at the lowest water content, wheras the other three moisture series do not differ much. An analysis (Table 3) of the differences in log M (M=mutation rate), similar to the above treatment of the sterility data, discloses no great significance for an influence of the sowing time. If such an influence is present, $M_a < M_b$ in the 0 % series, whereas $M_a > M_b$ at the highest water contents (low p value in the 100 % series after elimination of the deviating value at 2,500 r). The comparison of seed moistures at the moment of irradiation reveals, as expected from Fig. 3 and Table 2 (due to the linear dose dependence about the same t and p values are obtained if relative mutation rates are compared), a highly significant difference between the 0 and 30 % series, the latter not being separate from the 100 % and untreated series.

In the neutron series (Fig. 4) most points are situated on that part of the curve where elimination phenomena cause a deviation from rectilinearity, but from the random order of the points it can be concluded with considerable assurance that the seed moisture exerts no effect on the mutation rate.

The frequencies of cells with chromosomal aberrations in the first root mitoses are illustrated in Fig. 5 (X-ray series) and Table 4 (neutron series; the doses — *cf.* Fig. 2 — grouped as medium and high). The

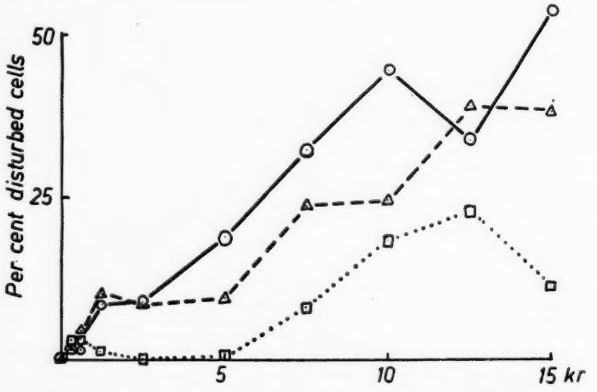


Fig. 5. Frequencies of cells, after X-irradiation, exhibiting chromosomal aberrations at the first root mitoses. Symbols as in Fig. 2. Abscissa: dose.

effect, at least at higher X-ray doses ($\geq$ 5,000 r), decreases in the order 0 % > 30 % > 100 %. Due to the small material investigated — only 70—350 mitoses in 3—12 roots being investigated in each sample — the somewhat peculiar »maxima» at low doses should not be regarded as significant. In the neutron series no differences between the moisture contents can be demonstrated.

Germination frequency and *survival* show, in the X-ray series, the

TABLE 4. *Frequency of cells with fragments and bridges at first mitoses in roots of seedlings, after irradiation of seeds with fast neutrons. Standard errors of frequencies estimated as* $\sqrt{\frac{P(1-P)}{n}}$, *P being frequency, n number of cells investigated.* (*Analysis performed by* O. GELIN.)

Conditions of seeds at irradiation	No. of investigated cells (n) and frequency of disturbed cells, in per cent			
	Low dose-group		High dose-group	
	n	100 ($P \pm m$)	n	100 ($P \pm m$)
Equilibrium with:				
0 % humidity	218	28,9 ± 3,1	307	35,8 ± 2,7
30 » »	228	30,7 ± 3,1	275	36,0 ± 2,9
100 » »	137	35,0 ± 4,1	112	28,6 ± 4,3

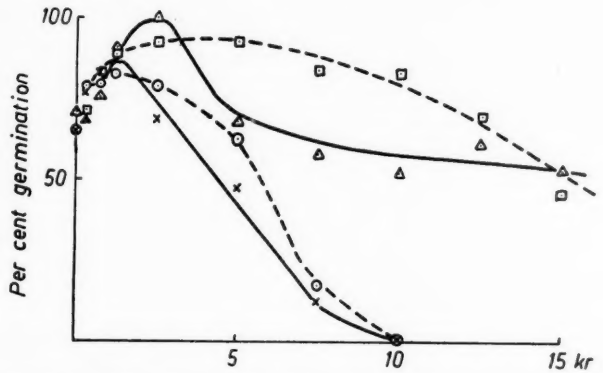


Fig. 6. Seedlings in per cent of seeds sown, at different X-ray doses. 0 % series, replication *a*: ×, b: ⊙; and 100 % series, replication *a*: △, *b*: ⊡. The curves from 30 % and untreated series not drawn. They take a position between those given; *cf.* Fig. 7.

same general dose dependence (Figs. 6 and 7): A fairly low germinability (about 70 %) and, in consequence, survival at the stage of maturity (about 60 %) is, in all series, increased at low doses. After passing a maximum, which broadens with increasing degree of seed moisture at the time of irradiation, the frequency of seedlings and mature plants decreases with increasing doses. The influences of seed moisture and sowing time are no doubt highly significant, but due to the peculiar shape of the curves, any mathematical expression for the radiation effect must be rather arbitrary. In Table 5 are interpolated, from the curves, the doses causing a reduction of germination and survival to 50 % (LD_{50}) and 30 % (LD_{70}), the latter being about one-half of an average control value. It is demonstrated, as was already shown in the diagrams, that in each replication the lethalizing action of the X-rays decreases in the order 0 % > 30 % ~ untreated > 100 %; further that the differences between the *a* and *b* replications — the injury being greatest in the series sown first — are appreciable at medium doses but become smaller, or even possibly reversed, at high doses.

In order to obtain a measure of the dependence of the radiation effectiveness on the seed moisture, the differences, *d*, between the logarithms of the induced lethalities, *L*, were analysed. To obviate overestimation of the differences, *L* was defined as high as (100 — per cent survival at maturity), *i. e.*, the more or less fortuitous germination inhibition in

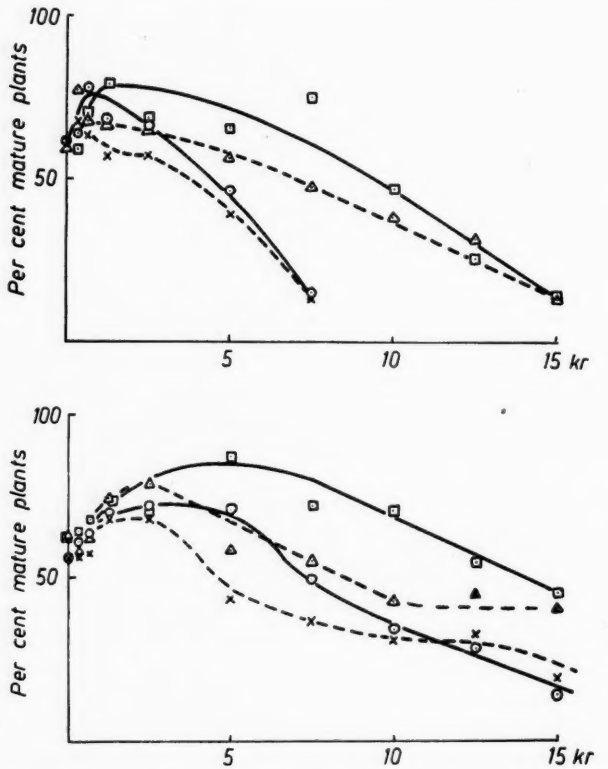


Fig. 7. Survival at maturity, per cent of seeds sown, at different X-ray doses. Upper diagram: 0 % series, replication *a*: ×, *b*: ⊙; and untreated series, replication *a*: △, *b*: ⊡. — Lower digram: 30 % series, replication *a*: ×, *b*: ⊙, and 100 % series, replication *a*: △, and *b*: ⊡.

the control samples (*cf.* p. 143) was disregarded, and the ideal, 100 %, used as a control value. Only the descending limb of the curve was analysed. The analysis is justified, since the d values thus determined do not vary with the dose, although the fact that the 100 % series is the one most affected by the sowing time, makes d — *e. g.*, in the comparison 30 %—100 % — smaller in the *a* than in the *b* replication; *cf.* Table 6. A fairly high degree of significance is found for the differences determined, except for $d = \log L_{30\,\%} - \log L_{\text{untr.}}$ Since in all moisture series $d = \log L_a - \log L_b$ varied with the dose (*cf.* Table 5), these differ-

TABLE 5. *Interpolated doses reducing the germination and survival frequencies to 50 % (LD_{50}) and 30 % (LD_{70}).*

Humidity of air in equilibrium with seeds at irradiation	LD_{50}, kr		Dose reduction (a/b)	LD_{70}, kr		Dose reduction (a/b)
	a series	*b* series		*a* series	*b* series	
			Germination:			
0 %	4,5	5,7	0,79	6,9	6,7	0,90
30 %	6,2	9,2	0,67	11,5	12,2	0,94
untreated	7,5	11,2	0,67	13,5	13,5	1
100 %	10—15	~15	(≤1)	>15	>15	—
			Survival:			
0 %	3,7	4,5	0,82	6,9	6,4	0,94
30 %	~5	7,5	0,7	10—12,5	11,5	~1
untreated	7,0	9,5	0,74	11,5	12,5	0,92
100 %	8,5	14,0	0,61	~18[1]	~18[1]	~1

[1] Extrapolated.

TABLE 6. *Comparison of differences between logarithms of induced lethalities ($L=100$ — per cent survival), seeds of different water content being irradiated.*

Comparison	$\bar{d}$ (see text)	m	n	t	p
0 % — 30 %	0,165	0,034	6	4,9	0,001 — 0,01
30 % — untreated	0,054	0,034	10	1,6	0,1 — 0,2
30 % — 100 %	0,193	0,030	10	6,4	<0,001
30 % — 100 %; (*a*-series)	0,120	0,044	5	2,7	~0,05
30 % — 100 %; (*b*-series)	0,265	0,036	5	7,4	0,001 — 0,01

ences could not be investigated, as was done with the sterility and mutation data.

In the neutron series a low lethalizing effect at doses giving high sterilities and mutation rates was evident, in accordance with several earlier experiments (*cf.* EHRENBERG and SAELAND, 1954, and literature quoted). The survival frequencies were thus about constant and equal to the control value up to the highest doses, about 1,000 rep, where a lethalizing action could be detected (*cf.* EHRENBERG and NYBOM, 1954).

DISCUSSION

1. *Seed Moisture and Radiation Effects.* — Within the whole moisture range investigated — 8,6—18,1 % H_2O — it was found that the

effectiveness of X-rays decreases with increasing water content, with respect both to induced lethality and to sterility. Due to the similarity of the survival curves at the seedling and maturity stages, it can be concluded that most of the lethality is caused at an early stage, before determination of the germination frequency (*cf.* EHRENBERG and NYBOM, 1954). When, on the other hand, the material is irradiated with fast neutrons, the effects are not found to depend on the moisture of the seeds. It can also be observed that, at least at higher doses, the frequency of chromosomal disturbances is influenced in a similar manner by the water content at the moment of irradiation (Fig. 5; Table 4). So far, the results accord with earlier findings in studies of growth inhibition (EHRENBERG, 1955 a). The different effects cannot be compared quantitatively, because of the arbitrary scale used to express the lethality induced, and because of the different germination conditions under which chromosome disturbances were determined. Bearing in mind that the numerical values for the lethalities are somewhat underestimated, it can be said, however, that changes of the water content cause changes of the same order of magnitude as regards lethality and sterility (*cf.* $\bar{d}$ values for corresponding comparisons in Tables 1 and 6).

The influence of seed moisture on the mutagenic effect of X-rays seems, on the other hand, to be limited to low water contents (8,6—10,5 % H_2O); only $\bar{d}_{0-30\,\%}$ in Table 3 differs significantly from zero. A comparison of corresponding effectiveness differences in the mutation and sterility analyses is permissible, since both effects are measured exactly. The $\bar{d}_{0-30\,\%}$ are almost identical for the two effects (*cf.* Tables 1 and 3), corresponding to an effectiveness reduction of 2,3 times. In order to test, independently of an influence of the dose, whether mutation rate and sterility depend to a different degree on the water content in the region $> 10{,}5$ %, the differences $D_{S-M} = d_S - d_M$ were determined. Here d_S and d_M equal the differences, $d_{30-100\,\%}$, between log sterilities and between log mutation rates, respectively, determined at the same dose and replication, in the region 625—12,500 r. The mean, $\bar{D}_{S-M}$, is $0{,}154 \pm 0{,}0377$, which, with 13 degrees of freedom, gives $t = 4{,}1$, $0{,}01 > p > 0{,}001$. These results strongly suggest that high moisture contents have a greater influence on the sterility than on the mutation rate.

A similar analysis could be made of $D_{L-M} = d_L - d_M$, d_L being $d_{30-100\,\%}$ between log lethalities, determined on the same samples as d_M The mean, $\bar{D}_{L-M} = 0{,}199 \pm 0{,}0524$, gives, with 7 degrees of freedom (only 5,000—12,500 r included in the analysis), $t = 3{,}8$, $0{,}01 > p > 0{,}001$. Remembering that the d_M are arbitrary and underestimated, it can be con-

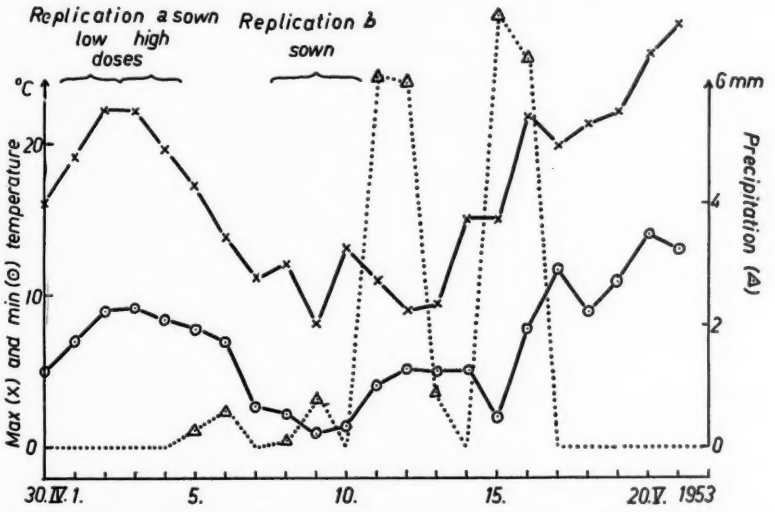


Fig. 8. Daily maximum and minimum temperature and precipitation during the germination of the seeds. Data from Lund, near the experimental field.

cluded that the water content exerts, on the induced lethality, too, a greater influence than on the mutation rate.

At high moisture contents of the seeds X-rays have been found to induce growth retardation more effectively than γ-rays (EHRENBERG, 1955 a). In consequence, the action of X-rays was found to be less dependent than that of γ-rays on the water content of seeds. In contrast to γ-radiation, a small fraction of the X-ray energy is dissipated at high ion densities ($\sim$ 200 ion pairs/μ; *cf.* SPIERS, 1952). The biological effectiveness of this fraction, analogously with that of neutron energy, may be expected to be independent of the water content. A possible explanation of the differing dependence of X-ray and γ-ray effects on the seed moisture is that, in the case of the former radiation, the densely ionizing fraction of the energy becomes relatively more effective with increasing water content.

A conceivable explanation of the present results would accordingly be that within the whole range of moisture contents investigated, sterility and lethality are, at least partly, effects of the sparsely ionizing fraction of the X-ray energy; whereas for the production of mutations, complete protection against sparse-ionization effects will be present at high

TABLE 7. *1954 experiment. Sterilities after germination at different temperatures.*

State of seeds at irradiation	Dose r	Mean spike fertility, $\bar{F}$, ± standard error; per cent germ. at 0°—10°C.	germ. at 10°—20°C.	Difference, $\bar{F}_{cold}-\bar{F}_{warmth}$	Probability p
Equilibrium	0	96,1 ± 0,65	97,8 ± 0,54	—1,7 ± 0,84	$p \sim 0{,}05$
with 0 %	2,500	89,0 ± 1,16	83,9 ± 1,75	5,1 ± 2,10	$0{,}02 > p > 0{,}01$
humidity	5,000	74,6 ± 1,83	65,6 ± 2,79	9,0 ± 3,34	$0{,}01 > p > 0{,}001$
	7,500	58,6 ± 1,91	83,0 ± 2,73	—24,4 ± 3,33	$p \sim 0$
Equilibrium	0	96,9 ± 0,79	98,1 ± 0,31	—1,2 ± 0,85	$0{,}2 > p > 0{,}1$
with 100 %	7,500	91,4 ± 1,12	90,7 ± 1,78	0,7 ± 2,10	$0{,}8 > p > 0{,}7$
humidity	12,500	82,4 ± 1,62	77,5 ± 1,92	4,9 ± 2,51	$p \sim 0{,}05$
	17,000	70,0 ± 2,00	69,0 ± 2,11	1,0 ± 2,92	$0{,}8 > p > 0{,}7$
Seeds soaked	0	93,5 ± 1,61	97,6 ± 0,38	—4,1 ± 1,65	$0{,}02 > p > 0{,}01$
24 hr at 20°	625	78,0 ± 1,61	75,5 ± 2,07	2,5 ± 2,62	$0{,}4 > p > 0{,}3$
	1,250	72,5 ± 3,11	75,4 ± 3,25	—2,9 ± 4,5	$p \sim 0{,}5$
	2,500	All plants killed			

water contents, with the effects of the small densely ionizing fraction predominating.

Irrespective of whether this or some other explanation is correct, it is interesting to note that the material back-ground of sterility (probably chiefly chromosomal aberrations) behaves differently to that of mutation.

2. *Influence of Germination Temperature.* — The weather conditions at Lund, near the experimental field, during the germination and first growth of the material are shown in Fig. 8. Having regard to the influence of germination temperature on seedling growth, the lower survival (Table 5) in the replication first sown, *a*, is probably connected causally with the low temperature during the week after sowing. Shortly after the sowing of replication *b*, a spell of hot weather occurred. The smaller differences between *a* and *b* at high doses might be ascribed to a storage effect, or to the fact that the 12,500 and 15,000 r series of replication *a* were sown later (*cf.* Fig. 8). The induced sterilities exhibited, at low moisture content, the reverse influence of the sowing time, whereas no influence was indicated in the 100 % series (Table 1). With a low degree of significance a similar influence could be detected in the mutation rates (Table 3): a negative $\bar{d}_{a-b}$ in the 0 % series, and $d_{a-b} \geq 0$ at the other water contents.

TABLE 8. *Distribution of mutation types within the different moisture series. Irradiation with X-rays and neutrons. Since in the X-ray material χ^2 for heterogeneity of replications gave $0{,}3 < p < 0{,}4$, the numbers from replications are lumped.*

Moisture series	Number of mutants of type: (in brackets per cent of total) albina	viridis	xantha	»rare»	Sum
X-rays:					
0 %	161	93	20	26	300
	(53,6)	(31,0)	(6,7)	(8,7)	
30 %	170	117	20	36	343
	(49,6)	(34,1)	(5,8)	(10,5)	
100 %	284	188	25	55	552
	(51,4)	(34,1)	(4,5)	(10,0)	
untreated	166	126	19	31	342
	(48,5)	(36,8)	(5,6)	(9,1)	
Sum X-rays:	781	524	84	148	1,537
	(50,8)	(34,1)	(5,5)	(9,6)	
neutrons:					
0 %	87	58	8	12	165
	(52,7)	(35,2)	(4,8)	(7,3)	
30 %	99	75	17	25	216
	(45,8)	(34,7)	(7,9)	(11,6)	
100 %	108	61	14	19	202
	(53,5)	(30,2)	(6,9)	(9,4)	
untreated	129	81	11	20	241
	(53,5)	(33,6)	(4,6)	(8,3)	
Sum neutrons:	423	275	50	76	824
	(51,4)	(33,4)	(6,1)	(9,2)	

As regards these sterility data, the 1954 experiment with simultaneous sowing of the material in forcing-beds at two temperatures, makes a temperature influence, not a storage effect, probable as the cause of the difference. In the 0 % series (Table 7) it is strongly indicated that $\overline{F}_{\text{cold}} > \overline{F}_{\text{warmth}}$, *i. e.*, $\overline{S}_{\text{cold}} < \overline{S}_{\text{warmth}}$. (At the highest dose, 7,500 r, a marked elimination of sterility becomes evident, as has been found repeatedly; *cf.* EHRENBERG *et al.*, 1952). In further agreement with the 1953 experiment, no corresponding difference is probable in the 100 % series. The same is the case with soaked seeds, which, in experiments involving measurements of growth retardation, were not influenced by the germination temperature (EHRENBERG, 1955 a).

Further experiments are necessary to settle whether the temperature influence on radiation induced sterility, which is the reverse of that

found for chromosomal disturbances (GELIN, 1953), lethality, and growth inhibition, is due to the induction, at the lower temperature, of a lower frequency of changes giving rise to sterility, or to a more effective elimination of such changes (by reunion of broken chromosome ends or elimination of injured cells or cell groups).

3. *Relation between Observed and Primary Changes: Distribution of Mutation Types.* — When discussing results of the type presented here it should be remembered that very little is known about the primary chemical changes which lead ultimately to the observed effects: lethality, sterility, or mutation. Each of these end effects might have several causes, with a different dependence on the various factors.

The mutation rate was here estimated as the frequency of spikes whose seeds segregate for plastid and pigment disturbances (for the sake of simplicity, often denoted as »chlorophyll mutations»). From their colour, the mutants are divided into type groups (*cf.* HOLM, 1954), each of which certainly represents changes in several loci. GUSTAFSSON (1940, 1947) described the types as albina, viridis, and xantha, which are rather common, and alboviridis, tigrina, *etc.*, the latter group being treated here as »rare» mutants. An analysis of the distribution of these mutant types (Table 8) demonstrates that, if we calculate with sums obtained at all X-ray doses, sowing time (germination temperature) and seed moisture at irradiation exert no influence on the distribution. As to seed moisture (sowing time not investigated), this applies also to the neutron series; χ^2 for heterogeneity gives, for both radiations, $p > 0{,}5$.

Nor is any difference found between the neutron and X-ray series. This fact is interesting, since there is strong evidence that the frequency of rare mutants depends on the ion density (*cf.* EHRENBERG and SAELAND, 1954 b; EHRENBERG and ANDERSSON, 1954), when a wide range of ion densities is studied, from 8 (γ-rays) up to 3,700 ion pairs/μ (Rn α-rays). Compared to this range, the difference between the ion densities of X-rays ($\sim$100) and fast neutrons ($\sim$400 ion pairs/μ) is small, but the fact that most ion density dependent effects are changed in the range around 200 ion pairs/μ (GRAY, 1954; *cf.* BONÉT-MAURY, 1952) suggests that for the production of (at least some of) the rare mutants another kind of dependence on ion density is present.

In the present material the distribution of mutant types seems to be displaced, the frequency of viridis mutations mounting, with increasing dose. In Table 9 the material has been divided into two dose ranges: a »low» ($\leq$ 5,000 r) and a »high» dose range ($>$ 5,000 r). From the statistical analysis the following conclusions can be drawn: Within moisture series and dose ranges, no heterogeneity of replications is in-

TABLE 9. *Number of mutants, classified as viridis and »others», in the different moisture series and dose ranges.*

Dose range	replication (*a*, *b*)	0 % viridis	0 % others	30 % viridis	30 % others	untreated viridis	untreated others	100 % viridis	100 % others
low doses	*a*	35	92	25	40	23	58	13	51
(313—5,000 r)	*b*	43	106	18	56	21	39	14	44
$\Sigma\,(a+b)$, per cent viridis		28,3		31,6		31,2		22,2	
Total:		viridis: 192 (28,3 %); others: 486							
high doses	*a*	10	3	38	70	43	62	85	146
(7,500—15,000 r)	*b*	5	6	36	60	39	57	76	123
$\Sigma\,(a+b)$, per cent viridis		(62,5)		36,3		40,8		37,5	
Total:		viridis: 332 (38,6 %); others: 527							

Comparison	χ^2 for heterogeneity	degrees of freedom	*p*
1) Between replications, within moisture series and dose ranges	6,98	8	0,5 — 0,7
2) a) Between moisture series within dose ranges	10,24	6	~ 0,1
b) Between 30 % + untreated series and 100 % series, within low dose range	3,34	1	0,05 — 0,10
c) Between 0 % series and other series within high dose class	5,92	1	0,01 — 0,02
3) Between dose ranges within			
a) 0 % series	12,10	1	< 0,001
b) 30 % series	1,05	1	~ 0,30
c) untr. series	3,28	1	0,05 — 0,10
d) χ^2 for sum 30 % + untreated:	4,01	1	0,02 — 0,05
e) 100 % series	9,92	1	0,01 — 0,001
f) χ^2 for sum 30 % + untreated + 100 %	11,03	1	< 0,001
g) Sum of χ^2 for all moisture series	26,35	4	< 0,001
h) χ^2 for sum of all moisture series	18,00	1	< 0,001
i) Difference: heterogeneity of series	8,35	3	0,02 — 0,05
k) Part of this heterogeneity possibly due to difference between 30 % + untr. series and 100 % series:	2,90	1	0,05 — 0,10

dicated (comparison 1). Hence the further analysis can be based on the sums of the numbers obtained in *a* and *b*. In the statistical treatment some circumspection is necessary due to the low numbers in the 0 % series at high doses, where the high viridis frequency is not definitely representative. However, χ^2 for the sum frequencies strongly suggests heterogeneity (Table 9: 3 h), even when the 0 % series is excluded

(Table 9: 3 f). The suggestion of heterogeneity, as to the reaction to a dose increase, of moisture series (Table 9: 3 i) might possibly be ascribed to the low viridis frequency in the 100 % series (comparisons 2 b, 3 k), though a great deal of χ^2 for heterogeneity between moisture series has to be attributed to an influence of the 0 % series at high doses (*cf.* 2 c and 3 a in Table 9).

The dose dependence of the (relative) frequency of viridis mutants accords with earlier indications (GUTAFSSON, 1947) that this type of mutation is obtained chiefly in combination with high sterilities. That no simple relation to induced sterility is present appears, however, from the fact that within the low-dose range, the viridis frequency is not higher in the 0 % series than in the 30 % + untreated series, although the sterility induced in the former is about 2,3 times greater. Within this range of water contents all mutation types show the same dependence on the water content as does the sterility. The lower viridis frequency in the 100 % series, as indicated in Table 9: comparisons 2 b, 3 k, is of interest, since within this moisture range the total mutation frequency was found to be independent of the water content (*cf.* Table 3). If this deviating viridis frequency is a real one, the consequence will be that within the whole range of moisture contents the absolute rate of viridis mutations exhibits the same quantitative dependence on the water content as does the sterility (*cf.* Table 1). The deviating dependence, at high water contents, of the »other» mutations will then be even more evident. The heterogeneities indicated within what has been summarily called the »mutation rate» necessitates some circumspection when different radiation effects are compared: An observed end effect has been shown to be caused by different primary reactions, which depend in a different manner on applied variables. Within an observed sum effect several influences might compensate and therefore partially mask each other.

4. *Theoretical interpretations* of data cannot be given, at the present stage of experiments, beyond the suggestions given above and in the discussion of the influences of water content and germination temperature on the radiation induced growth inhibition (EHRENBERG, 1955 a). The relation between induced sterility and mutations should only be touched briefly. In the last-mentioned paper it was shown that the increase of variation of seedling heights after X-irradiation — as compared to control or neutron irradiated samples — can be explained by the variation of the water contents and, consequently, X-ray sensitiv-

ities of individual seeds. In line with GUSTAFSSON's (1947) findings for most mutation types, later studies have given evidence that in some experiments, after X-irradiation, the fertility of mutated spikes (or plants) is significantly lower than the average fertility of the sample, whereas, after neutron irradiation, the reverse or no difference is found. In the present investigation it was shown that, in the region of low water contents, *i. e.*, around the average, 10 % H_2O, of the untreated samples mostly used, mutation rate and sterility depend about equally on the water content. A correlative occurrence of mutations and sterility can therefore be expected, exceptionally dry seeds being most sensitive to the induction of both effects. In neutron experiments, where the effects are not influenced by the water content, mutation and sterility occur independently, if not a reverse relation is induced by an elimination of mutation cases at the highest sterilities (*cf.* Fig. 4). Especially at high doses a dominating influence of the elimination may cause a negative correlation between mutation and sterility in X-ray series, too (*cf.* NYBOM *et al.*, 1953). The fact that the average fertilities of mutated spikes segregating for different mutation types, are different, might at least partially be explained by a different dependence of mutation types on the water content, as was indicated in Table 9. A general paper on these problems will be published shortly, in co-operation with GUSTAFSSON and NYBOM.

5. *The »stimulation» of the germination*, caused by low X-ray doses (Figs. 6 and 7) should be discussed briefly. In field experiments, 80—90 per cent of the seeds normally germinate, though sometimes a lower frequency is obtained. In the latter cases, the decreased germinability is often, without special investigations, ascribed to bad weather conditions after sowing, *e. g.*, a long period of drought interrupting the germination process at a stage with developed radicles. In such cases, treatment with certain chemicals having the ability to retard the germination process often leads to a higher germination and survival frequency in the treated than in the control samples; the former are exposed to the unfavourable conditions at an earlier, and hence less sensitive, stage of development. It is not impossible, of course, that the X-rays in the present case act in a manner similar to that of the chemicals mentioned, but this is contradicted by the fact that, in laboratory experiments, the germination process is not affected at all by radiation doses in the actual region.

The question whether ionizing radiation can stimulate plant growth at all has been much discussed: Some authors give data which demonstrate unequivocally that low X-ray doses increase germinability and dry matter production (SHULL and MITCHELL, 1933) as well as respiration rate (FRANCIS, 1934); whereas in many other experiments no similar effects whatsoever have been found; *cf.* JOHNSON's summary (1936). The contradictory findings might suggest that in the experimental materials used, certain radiosensitive inhibitory factors have been present in some cases and

absent in others. The germination inhibition might be induced by several, not yet defined, factors summarily described as unfavourable storage; the concept of secondary dormancy (*cf.* MEYER and ANDERSON, 1954) probably includes such cases.

Dormant *Syringa* buds, *i. e.*, those with decreased sprouting ability, can be forced with moderate doses of γ-rays (MOLISCH, 1912; *cf.* SKOOG, 1935). It has further been demonstrated that low radiation doses cause such changes of the plasm viscosity as are typical of true cell stimulation (VIRGIN and EHRENBERG, 1953). It seems probable, therefore, that the higher germinability after irradiation with low doses has to be ascribed to the breaking of some kind of inhibited condition, whether this is effected by a non-specific injury of the radiation or by the destruction of an inhibitory factor. Since the material used in the present investigation showed 100 per cent germinability in a parallel laboratory test, the influence of environmental factors during germination has to be considered, too. The (probably storage induced) inhibitory factor is certainly well differentiated from post-harvest unripeness (dormancy). In order to force the germination of barley in this condition, doses amounting to about $5 \cdot 10^6$ r must be applied (EHRENBERG, unpubl.); *i. e.*, doses causing total lethality, the plants dying 1—3 weeks after the start of germination.

Acknowledgment. — The field treatment and biological analysis of the present material were included in the routine mutation work at Lund and Svalöf, executed under the direction of Professor ÅKE GUSTAFSSON, Stockholm. I wish to express my sincere thanks to him and to the members of his staff, especially Dr. NILS NYBOM and Mrs. UDDA LUNDQVIST, Lund, for their time-consuming contributions to the work; and further to Professor ARNE MÜNTZING, who placed the facilities of the Institute of Genetics, Lund, at my disposal. The investigation was supported financially by Knut and Alice Wallenberg Foundation.

SUMMARY

(1) The lethality, sterility, and chromosomal disturbances provoked by X-irradiating barley seeds were found to decrease with increasing water content of the seeds, at the moment of irradiation, in the range of 8—18 % H_2O.

(2) The mutation rate induced, estimated as frequency of chlorophyll mutations, showed a parallel dependence on the moisture only at low contents ($\leq$ 11,5 % H_2O). Above 11,5 % H_2O the total mutation rate was found independent of the water content, although it is possible that mutation types differ in this respect.

(3) The effectiveness of fast neutrons to produce the effects studied was found independent of the seed moisture.

(4) After germinating X-irradiated seeds at a low temperature, a

higher lethality was developed, wheras the sterility — after irradiation of dried seeds ($\sim$ 8 % H_2O) — was lower.

Literature cited

BONÉT-MAURY, P. 1952. Chemical phenomena in irradiated pure water. — Discussions Faraday Soc. 12: 72—79.

BONNIER, G. and TEDIN, O. 1940. Biologisk variationsanalys. — Stockholm.

EHRENBERG, L. 1955 a. The radiation induced growth inhibition in seedlings. — Botan. Notiser 108: 184—215.

— 1955 b. Biological effectiveness of fast neutrons. — (In preparation.)

EHRENBERG, L. and ANDERSSON, G. 1954. Probable side-effect of nuclear reactions in the biological action of fast protons. — Nature 173: 1086.

EHRENBERG, L., GUSTAFSSON, Å., LUNDQVIST, U. and NYBOM, N. 1953. Irradiation effects, seed soaking and oxygen pressure in barley. — Hereditas XXXIX: 493—504.

EHRENBERG, L., GUSTAFSSON, Å. and NYBOM, N. 1952. Effects of ionizing radiations in barley. — Arkiv Botan. Ser. 2, 1, No. 17: 557—568. (*Cf.* EHRENBERG, L. 1951. Mutation studies with radioactive isotopes. — Radioisotope Techniques I. London 1953, pp. 452—461.)

EHRENBERG, L. and NYBOM, N. 1954. Ion density and biological effectiveness of radiations. — Acta Agr. Scand. 4: 396—418.

EHRENBERG, L. and SAELAND, E. 1954 a. Chemical dosimetry of radiations giving different ion densities. An experimental determination of G values for Fe^{2+} oxidation. — JENER Publ. No. 8: 1—25.

— and — 1954 b. Effects of pile radiation on barley seeds. — J. Nuclear Energy 1: 150—169.

FRANCIS, D. S. 1934. The effects of X-rays on growth and respiration of wheat seedlings. — Bull. Torrey Botan. Club 61: 119—153.

GELIN, O. E. V. 1953. Mitotische Störungsfrequenzen in röntgenbestrahlter Gerste. — Agri Hort. Genet. 11: 66—81.

GRAY, L. H. 1954. Some characteristics of biological damage induced by ionizing radiations. — Radiation Research 1: 189—213.

GUSTAFSSON, Å. 1940. The mutation system of the chlorophyll apparatus. — Lunds Univ. Årsskr., N. F., Avd. 2, 36: 1—40.

— 1947. Mutations in agricultural plants. — Hereditas XXXIII: 1—100.

HOLM, G. 1954. Chlorophyll mutations in barley. — Acta Agr. Scand. 4: 457—471.

JOHNSON, E. L. 1936. Effects of X-rays upon green plants. — In DUGGAR: Biological Effects of Radiation. Vol. II. New York and London, pp. 961—985.

MEYER, B. S. and ANDERSON, D. B. 1954. Plant Physiology. 2nd ed. — New York, Toronto, and London. (*Vide* p. 712.)

MOLISCH, H. 1912. Über das Treiben von Pflanzen mittels Radium. — Sitzungsber. Kgl. Akad. Wiss. Wien, Math.-Naturwiss. Klasse 121, Abt. 1: 121—138.

NYBOM, N. 1954. Mutation types in barley. — Acta Agr. Scand. 4: 430—456.

NYBOM, N., LUNDQVIST, U., GUSTAFSSON, Å. and EHRENBERG, L. 1953. Biological effects of X-irradiation at low temperatures. — Hereditas XXXIX: 445—457.

SHULL, C. A. and MITCHELL, J. W. 1933. Stimulative effects of X-rays on plant growth. — Plant Physiol. 8: 287—296.

SKOOG, F. 1935. The effect of X-irradiation on auxin and plant growth. — J. Cellular Comp. Physiol. 7: 227—270.

SPIERS, F. W. 1952. Radiation absorption and energy loss by primary and secondary particles. — Discussions Faraday Soc. 12: 13—24.

VIRGIN, H. I. and EHRENBERG, L. 1953. Effects of β- and γ-rays on the protoplasmic viscosity of *Helodea* cells. — Physiol. Plant. 6: 159—165.

A NUCLEO-CYTOPLASMIC ANOMALY IN DROSOPHILA MELANOGASTER CAUSING INCREASED SENSITIVITY TO ANAESTHETICS

By *BERTIL RASMUSON*

INSTITUTE OF GENETICS, UNIVERSITY OF STOCKHOLM

I. INTRODUCTION

THE occurrence of non-mendelian or cytoplasmic inheritance was discovered by CORRENS (1909) in *Mirabilis*, *Urtica* and *Lunaria* and by BAUR (1909) in *Pelargonium*. Ever since, the rôle of cytoplasm in heredity has attracted interest and has been studied especially in plants. At first this type of inheritance was thought to be quite independent of the genome (WINKLER, 1924; RHOADES, 1933).

In animals the cytoplasmic inheritance seems to be either less frequent or more difficult to analyse. GOLDSCHMIDT (1924, 1934) was the first to establish the occurrence of an interaction between genes and cytoplasm in his investigations concerning different local strains of *Lymantria dispar*. In 1927 KÜHN showed that the inheritance of pigmentation in two laboratory strains of *Habrobracon* was associated with the cytoplasm (KÜHN, 1927). KIKKAWA (1941) studied the egg colour in *Bombyx* and made similar observations, but assumed the explanation to be a maternal influence or predetermination.

In *Drosophila*, only two cases of cytoplasmic inheritance have been described. L'HÉRITIER and TEISSIER (1937) found in an experiment where CO_2 was used as anaesthetics, that one of their *Dr. melanogaster* stocks did not recover from anaesthesia, the flies being killed by the gas. This CO_2-sensitivity was shown to be inherited by the cytoplasm. It was for a long time supposed to be quite independent of the genome of the animals but also in this case it has been possible to establish a gene-dependance (GUILLEMAIN and PLUS, 1953). The other case is the »sex ratio»-phenomenon in *Dr. bifasciata* studied by MAGNI (1953) where the production of male offspring is depressed by a cytoplasmic factor.

The most penetrating analyses of the interaction between genes and

cytoplasm have been performed on microorganisms. In *Paramecium*, SONNEBORN (1951) and his collaborators have investigated the hereditary mechanisms of the killer-character, the antigenes and the different mating types, all of which depend on extra-nuclear factors as well as genes. In yeast EPHRUSSI and others have studied the co-operation of genes and cytoplasm in the metabolism (EPHRUSSI, 1953).

The present investigation concerns an outstanding sensitivity to anaesthetics found in a wild type strain of *Dr. melanogaster*, the inheritance of which seems to depend on cytoplasmic factors as well as genes.

This strain was found by chance in an investigation concerning selection for sternopleural bristle number. In counting the bristles it was found that one strain, originating from the wild type stock Örebro and selected for low number of sternopleural bristles, recovered from anaesthesia much more rapidly than any of the other strains used. At stronger etherization the flies from this strain also were easily killed. This sensitivity to ether was observed during many generations, and thus was inherited.

Speed of etherization and recovery from anaesthesia have previously been investigated by STERN, SCHAEFFER and SPENCER (1944) in an investigation concerning morphological and physiological differences between *Drosophila americana* and *Dr. virilis*. *Dr. americana* females were outcrossed to *Dr. virilis* males, and the *virilis* genome was introduced in the *americana* cytoplasm by repeated back-crosses during a period of 2 years. At the end of this period the flies from 3 parallel hybrid lines were found to agree with the *virilis* species in all respects, including the sensitivity to ether. The authors conclude that the differences between the two species »do not depend on any self-reproducing extra-chromosomal elements but on the genetic constitution of the chromosomes of the two species».

The results of the present investigation indicate that the sensitivity to anaesthetics in this case depends on an interaction between genes and cytoplasm. However the back-cross lines have been followed from the beginning and the conclusions drawn rest on observations from the earlier generations. If the analysis of the back-cross lines had been postponed until many generations later, as in the investigation by STERN *et al.*, it is quite possible that no evidence had remained of the extra-nuclear influence. The factors causing the sensitivity may also be quite different from those in the *americana-virilis*-case.

II. MATERIAL AND METHODS

When the investigation of the sensitive strain started it had been propagated for at least 20 generations in cultures of 4 animals and thus ought to be highly inbred. In most of the experiments this strain, which has been called S, was compared to an inbred strain of the wild type laboratory strain Florida. This strain was chosen as being genetically homogeneous and apparently normal in its reaction to anaesthetics. It has been called the N-strain.

1. Outline of culturing and collection of flies

To preserve the homozygosis of the N- and S-strains at as high a level as possible and to ascertain uniform material for the testings, a special culturing technique has been followed throughout the experiments. The outline is shown graphically in Fig. 1.

In each generation of the strains 6 pairs of flies were taken at random from the offspring of one pair-culture from the preceding generation. These 6 pairs were allowed to propagate separately in bottles containing standard culturing medium at $25° \pm 1°$ C. The pure strains were thus inbred by brother-sister mating in every generation.

For the tests of anaesthesia a great number of animals was needed, and the offspring from the 6 pair-cultures did not suffice, but had to be multiplied in each generation. For this purpose 18 new cultures were set up from these animals, each consisting of 3—4 pairs of parents. These cultures were always held synchronous with the crosses with which they were to be compared. When the offspring started to emerge, the bottles were emptied within certain time intervals in order to obtain animals of uniform age for the testings. Virgin females and young males were also collected for use in the next generations of back-crosses. After 3 days the bottles were discarded without further propagation.

Each generation of the back-cross lines consisted of 12 cultures with 3—4 pairs of parents each. The total number of parents was thus 36—48. When emergence had started the bottles were emptied in the morning and virgin females and young males for propagation were collected from this batch. During the day new batches were collected after certain intervals, and these animals were used in the tests. The bottles were discarded after 3 days, to avoid influences of crowding that might arise in old cultures.

No conscious selection has thus been practised either in the pure strains or in the back-cross lines. The pure strains have been kept

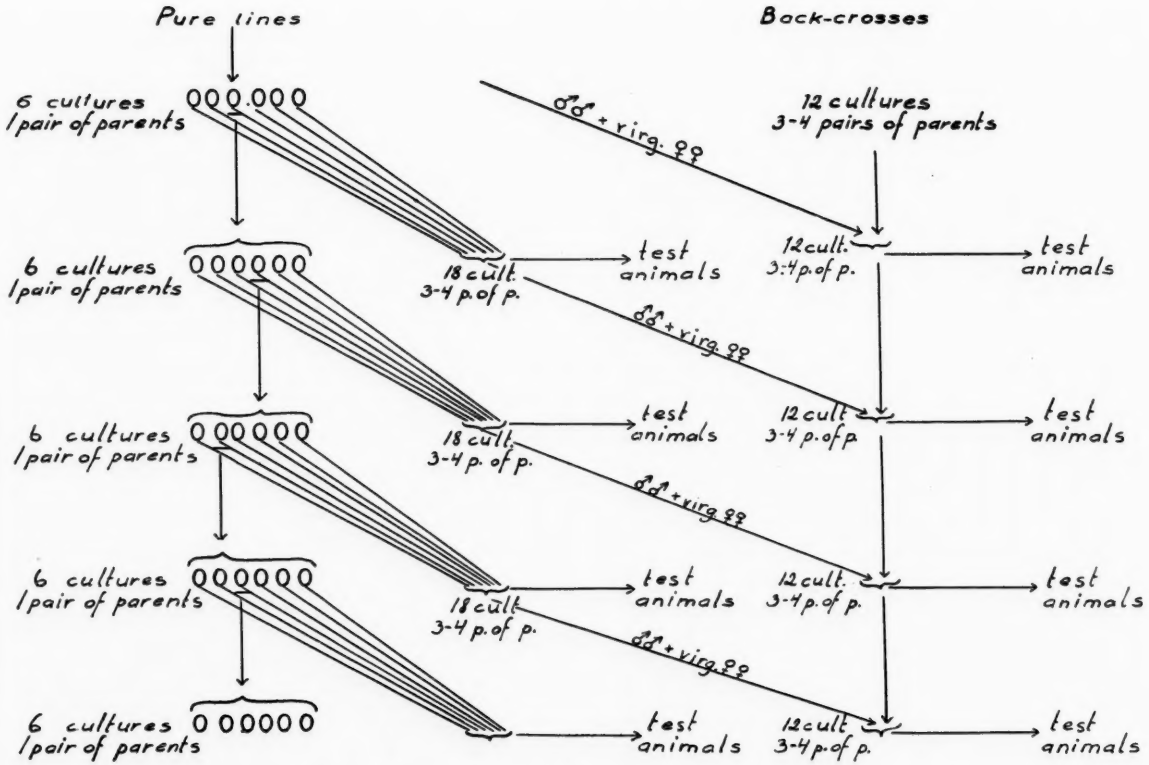


Fig. 1. Outline for culturing and collection of test animals.

constant by inbreeding, and the uniformity of the test animals is ascertained by their common origin from one pair of grandparents and by the use of only the first emerged animals which are the most uniform in size.

In experiments with flies of different ages the interval between collectings has varied. As the young animals show rapid changes in sensitivity with age, short intervals must be used in collection of animals which are to be tested young. 1 hr. was found to be the shortest interval possible if enough flies were to emerge. In animals older than 12—24 hr. the changes with age are small and the interval could thus be made longer. The following deviations from mean age have been allowed:

$3 \pm {}^1/_2$ hr.
6 ± 1 »
12 ± 2 »
24 and more ± 3 »

In part of the later experiments a somewhat different technique of culturing has been used. The animals have been cultured in vials supplied with a small block of standard medium placed on a cardboard strip. The blocks of food and the cardboard strips were changed each day and the old ones, where eggs had been deposited, were transferred to new vials containing cellulose moistened with a suspension of live yeast. The sensitivity of offspring from these cultures was compared to that of animals reared in bottles on standard medium in a number of tests, but no differences were found either for the pure strains or for cross-bred animals. The changed culturing technique thus had no effect on the sensitivity.

When the culture bottles or vials were emptied, the animals were transferred, without being anaesthetized, to vials supplied with standard medium and were kept there in 25° C until about 1 hr. before they had reached the proper age for testing.

2. Methods of testing the sensitivity to anaesthetics

A. Experimental apparatus

The sensitive strain was first recognized by its ability to recover from anaesthesia more rapidly than other wild type strains. It is, however, impossible to obtain a reliable estimate of the exact time of recovery, partly because nothing is known about the depth of anaesthesia for a

particular animal, and partly because recovery takes place step by step with frequent relapses.

It was, however, possible to approach the problem from another angle, as it was found that the sensitive strain was also more rapidly brought to immobility and death than other strains with the same dose of ether. The time taken to attain immobility or death was therefore used as a criterion of sensitivity in most of the experiments.

Great difficulties were met with in attempts to construct an apparatus where the dose of a gaseous anaesthetic could be held constant and could be repeated in successive experiments. The reliability of constructions following the principle of »constant flow» was tested with consistent groups of flies, the mean lethal times of which were compared. The

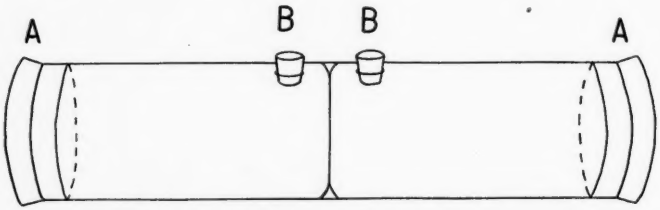


Fig. 2. Apparatus for testing the sensitivity to anaesthetics. (See text.)

best agreement between experiments was, however, reached with the simple apparatus shown in Fig. 2. It does not involve any gaseous flow but consists of a glass tube measuring 475 ml., both ends of which are closed by rubber stoppers (A). The tube is separated into two halves by a fine-meshed wire-netting which fits tightly into the tube. On both sides of the net a hole is bored in the tube wall (B).

An experiment is undertaken in the following way: About 1 hr. before the test, the animals which are of known age, are anaesthetized with divinyl ether. They are classified according to sex and divided into groups of about 20 animals of the same sex which are transferred to small vials (the openings of which fit into the holes B in the glass tube) where they are allowed to recover from anaesthesia.

Immediately before the test the glass tube is closed by the rubber stoppers A and also by a smaller stopper in one of the holes B. A certain amount of the anaesthetic is injected by means of a Record-syringe through the other hole B, which is then closed by a stopper, pierced with a fine capillary. When evaporation of the anaesthetic is complete the

pierced stopper is replaced by a tight one, so that the tube becomes completely closed.

The tests are made with two groups of flies simultaneously. Two vials containing the assorted and recovered flies are put into a spring-holder where they are fixed at the same distance as that between the two holes B. The small stoppers in these are removed, the flies are rapidly introduced through the holes, which are then immediately reclosed. At the same time a stop-watch is started. Every 15 seconds a note is made of the number of flies which are motionless or dead, and from these observations a mean reaction time is calculated.

Between tests the tube has always been left open and the same set of stoppers A has not been used in succeeding tests to insure that all anaesthetic from the preceding test has vanished before a new one is started. The temperature of the room has been kept at 20°—21° C.

In some tests which will be described later the mean effective dose (ED50) was sought instead of the mean time of reaction. To be fit for use in these tests the tube must be proved to hold a constant concentration for at least 2 hr. The following test was made: The moment of death was observed for a group of flies in one half of the tube, which was then left closed. After 2 hr. another test was made in the other half of the tube with flies of the same age as in the first test. No significant difference was observed between the two tests and the tube could therefore be used for tests of longer duration.

B. The dose

A high concentration of anaesthetic rapidly brings the exposed animals to immobility and death. There is also a minimum concentration below which no anaesthetic effect is produced no matter how long exposure is continued. Because of this graded relationship between dose and response, a suitable concentration of anaesthetic has been worked out, which in the following experiments has been used in all tests of cross-bred flies. As the range tends to increase with increased mean time the concentration ought not to be too low; if it is too high the range becomes so small that the moment of reaction for the individual fly cannot be exactly registered. The dose has been fixed so as to make it possible to classify the flies exactly during the period of reaction, *i. e.*, the time between the moment when the first fly in the group is observed to react and the moment when the last fly reacts.

The two observations possible in these tests are: (1) The moment

when the anaesthetic has brought the depression of action in the central nervous system to the degree that immobility will occur, (2) the moment of death. One or both of these observations have been registered in the tests, depending on the current problem. It ought, however, to be noted that in all the tests of flies from the back-cross experiments the moment of death has been used. This is because observations of commencing anaesthesia require a very low dose if all the flies are not to react simultaneously, and such low doses have the disadvantage that some individuals may lie motionless at one moment, but suddenly recover temporarily and walk around for a short period. The concentration of anaesthetic in the central nervous system seems to fluctuate about the threshold where the motility becomes impossible. Exact observations of this reaction are thus difficult to obtain.

The »moment of death» has been defined as the moment when the flies, which at rest and under anaesthesia keep their wings in a caudal position, fold the wings upwards over their back in an attitude similar to the resting position of butterflies. This moment does not exactly correspond to real death, which occurs a few seconds later, after which interval no recovery was ever observed (verified in several experiments with flies of different genotypes). The changed attitude of the wings might therefore be taken as a reliable criterion of death.

In the tests diethyl ether (Ph. Sued. Ed. XI: Aether ad narcosin) has been used. The injected amount of ether is calculated to give a concentration in the tube of about 5 % of volume in the observations of commencing anaesthesia (0,1 ml. injected) and of at least 30 % of volume in the observations of moment of death (1 ml. injected).

C. Sources of error

Although temperature and concentration of anaesthetic have been held as constant as possible, there are still a number of variable factors which may influence the tests, especially as the experiments must of necessity be stretched over a long period of time. Such factors are, for example, fluctuations in the condition of the flies, air-pressure and humidity. The condition of the flies has been kept as uniform as possible through the above-mentioned culturing technique. Air-pressure and humidity have been recorded at every testing.

Taken together all these factors add to the variation between repeated tests with flies of the same age and genotype. As expected, it was found that variation was less between simultaneously tested groups than between groups from different tests. This also indicates that the dose is

not quite constant. To avoid as many as possible of these sources of variation all the tests of cross-bred flies were made simultaneously with a group of control animals (S or N) of the same age in the other half of the glass tube. It thus became possible to use the mean difference from control in the analysis in addition to the real mean time of reaction.

The advantage of this method rests on the assumption that different genotypes react in the same way to the fluctuations in external conditions. To prove this, a statistical analysis was made on values from the back-cross line B, generations 4—11, following the formula given by FISHER (1925).

$$t = \frac{(b - \beta) \cdot \sqrt{S(x - \bar{x})^2}}{s}; \quad \beta = 1; \quad s = \sqrt{\frac{S(y - Y)^2}{n - 2}}$$

where the t-value gives the probability of deviation from the expected regression coefficient β. The analysis gave a value of P=0,1—0,2 and thus did not indicate any different reaction to environmental changes.

III. EXPERIMENTS INVOLVING PURE STRAINS

It was soon recognized that the mean time of reaction varied with varying age of the tested flies. The variation between tests was decreased when flies of uniform age were used.

To investigate the age dependency more thoroughly, tests of moment of death were made with flies (females) of known age from the strains N and S. As two groups could be tested simultaneously the following scheme was followed to avoid variation in dose (that may at that time have been rather great due to lack of routine):

Pairs of simultaneously tested groups:

S 3 hr. — S 6 hr.
S 6 hr. — N 6 hr.
N 6 hr. — N 3 hr.
N 3 hr. — S 3 hr.
S 6 hr. — S 12 hr.
S 12 hr. — N 12 hr.
N 12 hr. — N 6 hr.
N 6 hr. — S 6 hr.

and in the same way for flies of 12 and 24 hr. and of 24 and 60 hr. age. In this way all differences are covered without influence of dose variation. The results of these tests as to mean reaction time based on the

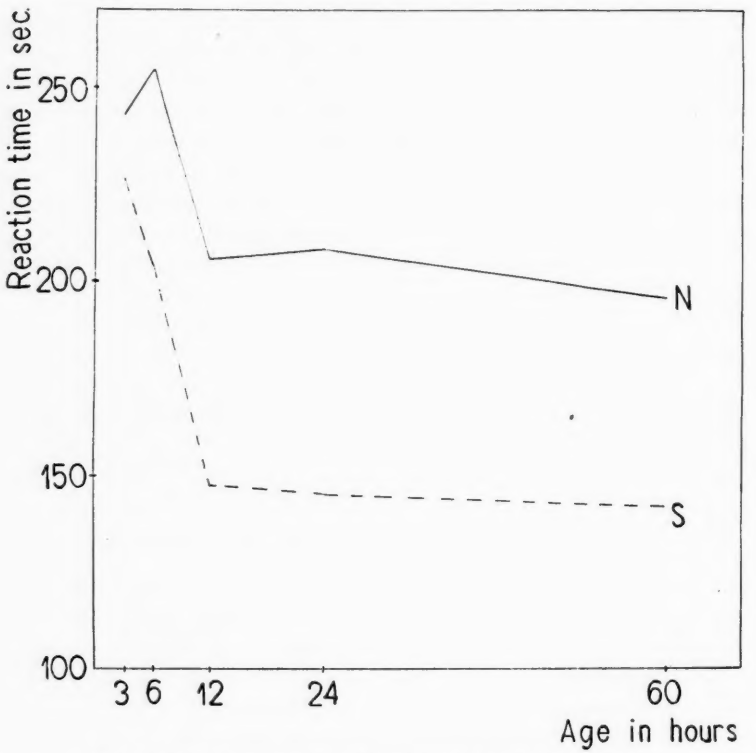


Fig. 3. Effect of ageing on the sensitivity of the pure strains.

total values (2 replications for the 3 hr.- and 60 hr.-groups, 3 replications for other age-groups) are given in Table 1 *a* and Fig. 3, the mean differences between simultaneously tested groups in Table 1 *b*.

The agreement between differences of total values and of simultaneous tests is good, so the errors due to method cannot have been too important. The difference between the two strains is least at the 3 hr. age, at 24 hr. it has reached its maximum value, and then decreases. This depends on the different reactions to ageing shown by the two strains. The resistance of the N-strain is high at 3 hr., increases still more up to 6 hr., shows a marked decline between 6 and 12 hr., keeps constant between 12 and 24 hr. and has further decreased at 60 hr. The S-strain is most resistant at 3 hr., already at 6 hr. the resistance is considerably decreased. Between 6 and 12 hr. the decline is also marked,

TABLE 1 *a. Mean reaction times for females of different age from the pure strains N and S. Each value is a mean of 2 or 3 non-simultaneous tests.*

Strain	Age				
	3 hr.	6 hr.	12 hr.	24 hr.	60 hr
N	243,40	255,46	206,94	207,66	195,75
S	226,72	204,37	147,04	145,68	142,15
Excess over S	16,68	51,09	59,90	61,98	53,60
Difference between ages	N —12,06	48,52	—0,72	11,91	
	S 22,35	57,33	1,36	3,53	

TABLE 1 *b. Differences between simultaneously tested groups. The significances of the differences are tested against a standard deviation of 13,61 sec. for differences between means of 20 animals derived from the mean square within tests of 8,231 (see Table 2).*

* $P < 0{,}05$; ** $P < 0{,}01$; *** $P < 0{,}001$; as in all the following tables.

Age	Differences N—S	Differences between ages	
		N	S
3 hr.	33,53*	—9,48	30,21*
6 hr.	45,54**	13,57	36,82*
12 hr.	63,34***	—3,90	2,67
24 hr.	71,25***	20,72	0,90
60 hr.	42,71***		

but from that age on the strain keeps constant. In both strains the young animals are thus the most resistant. The cause of this may be that the mechanism in the central nervous system that is blocked by the anaesthetic has not yet fully developed at eclosion. It is as yet, however, impossible to tell if there are any *general* physiological changes connected with the sensitivity to anaesthetics of the S-strain.

An analysis of variance based on all these tests gave significant differences between genotypes and between ages, as is shown in Table 2 *a*. There is also a significant difference between replications ascribable to

varying testing conditions. For obtaining the significance of the differences between simultaneously tested groups, therefore, the mean square within groups, 8,23 was used. This gave a standard deviation of 13,6 seconds for differences between means based on 20 animals, derived as $15\sqrt{\frac{2 \cdot 8{,}231}{20}}$, the class interval being 15 sec.

Here a special note ought to be made about the distribution of the reaction times of individual flies. The distribution within the groups indicated some skewness, but to test this statistically more extensive material was needed. As much more than 40—50 flies could not be tested simultaneously it was necessary to combine values from different tests. Then, however, the unavoidable dose-variation might assert itself and obscure the true distribution. The following method was therefore used in combining the values. In each test the mean reaction time was calculated, the interval of 15 sec. within which the mean came to lie was taken as the zero-value of a provisional scale with 15 seconds as scale unit. Adjusted to this provisional scale the values from different tests could then be added. Analysis of distribution were made on 1241 females from the S-strain and on 235 females from the N-strain used as controls in the back-cross lines. They gave the values for skewness (g_1 according to SNEDECOR, 1946, p. 174) $+0{,}431 \pm 0{,}069$ for S and $+0{,}565 \pm 0{,}159$ for N, both highly significant. The values for kurtosis (g_2 according to SNEDECOR) were $0{,}564 \pm 0{,}139$ for S and $0{,}368 \pm 0{,}316$ for N. The former is significant, the latter is not. It is thus clear that the distribution of the times of reaction is not normal, and this fact raises the question whether the usual z-test of significance is reliable or if some kind of transformation must be introduced.

To test this, the same analysis of variance that is shown in Table 2 *a* was repeated using the logarithmic values of the reaction times, as the log. transformation is known to decrease positive skewness. The result of this analysis is shown in Table 2 *b*. It appears from a comparison of *a* and *b* that the variance-ratios of the analysis with and without transformation are almost identical. No false conclusions can be drawn from the analysis of untransformed values. This was further confirmed in another comparison made on values from a back-cross line and it was therefore decided to use the untransformed values throughout the investigation.

At a later occasion the experiments involving pure strains were supplemented by tests of other wild type strains kept in the laboratory. The tests of reaction times were then complemented by weighing of the

TABLE 2 *a. Analysis of variance of mean reaction times for the pure strains N and S.*

Causes of variation	Degrees of freedom	Mean squares	Variance ratios
Differences between ages	4	501,407	1,48
Differences between N and S within ages	5	337,938	15,97***
Differences between repeated tests	16	21,167	2,57***
Differences within tests	518	8,231	
Differences between N and S	1	1684,107	6,70*
Differences between ages within strains	8	251,526	11,88***
Differences between repeated tests	16	21,167	

TABLE 2 *b. Analysis of variance after logarithmic transformation of the mean reaction times.*

Causes of variation	Degrees of freedom	Mean squares	Variance ratios
Differences between ages	4	0,5655	1,28
Differences between N and S within ages	5	0,4432	17,94***
Differences between repeated tests	16	0,0247	2,58***
Differences within tests	518	0,0096	
Differences between N and S	1	2,140	7,33*
Differences between ages within strains	8	0,2922	11,82***
Differences between repeated tests	16	0,0247	

TABLE 3. *Differences of mean reaction time between the S-strain and other wild type strains.*

Strain	Excess over S in seconds	No. of tests	Significances
Algeria	55,1	11	***
Canton S	57,0	8	**
Florida	81,4	4	P 0,02 — 0,01
Karsnäs	39,4	5	P 0,1 — 0,05
Oregon	51,7	10	**
Stäket	66,4	8	**
Tunnelgatan	68,3	5	P 0,02 — 0,1?

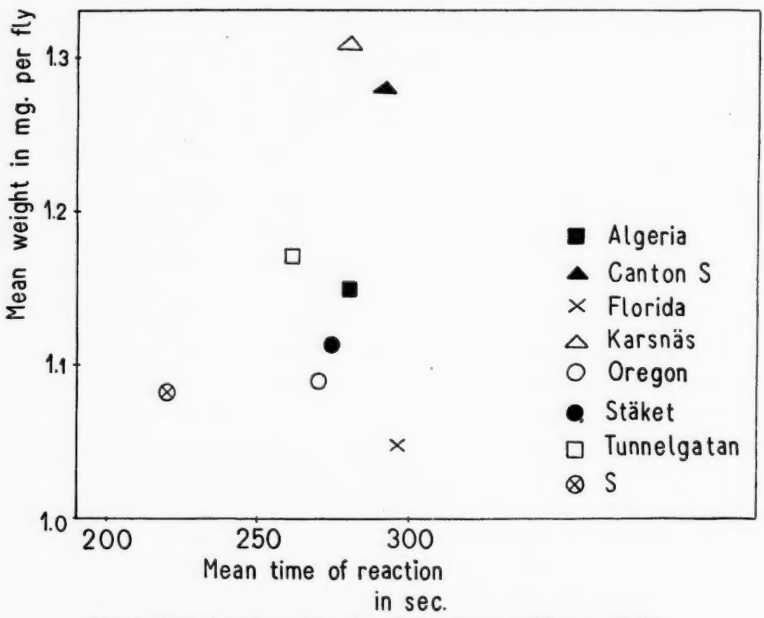


Fig. 4. Weight and reaction time for different wild type strains.

animals. 24 hr. old females from these strains were tested for moment of death against control groups from the S-strain of the same age. When tested, the batches were immediately weighed and the mean weight of the flies was calculated. The results of the tests of sensitivity are given in Table 3 and the correlation to weights in Fig. 4. The value for the S-strain in the figure is based on all the 51 control groups. It can be seen that although the mean weight varies within a large range all the strains except S agree well in mean time of reaction. There is no correlation between time of reaction and weight, and the cause of the divergent sensitivity of the S-strain must be searched for in some system that is not influenced by the body weight.

IV. THE SENSITIVITY OF THE OFFSPRING FROM RECIPROCAL CROSSES BETWEEN THE S-STRAIN AND DIFFERENT WILD TYPE STRAINS

The F_1 offspring from crosses between S and N were tested on different occasions, alone or in connection with the parental strains. The experiments were performed to investigate the following problems:

(1) Does the offspring from reciprocal crosses show any differences in resistance to anaesthetics? (2) Where do the reaction times of F_1 flies lie compared with those of the parents? (3) How do the reaction times of F_1 flies change with ageing?

The first of these questions was answered by testing reciprocal F_1 females, 24 hr. old in simultaneous tests. 9 such tests were made, giving the mean reaction times 301,61 sec. for N×S and 268,77 sec. for S×N (female parent always given first). The difference, 32,84 sec., was significant at the 0,001 % level.

The second question was investigated by testing F_1 females from reciprocal crosses against S-females. 12 tests were made on N×S, 14 on S×N. All the females were 24 hours old. The mean difference from S was 118,77 sec. for N×S and 90,09 sec. for S×N. The difference between the reciprocals was thus 28,68 seconds which is significant and agrees well with the preceding experiment. However, the mean reaction times for each type of fly, N×S 359,02 sec.; S×N 330,35 sec. are at a higher level than in the preceding experiment. As this holds also for the S-females (mean time 240,26 sec.) in comparison with earlier experiments, the discrepancy must be caused by environmental factors common to all the flies. An experiment analogous to this was also performed with F_1 males of the same age. They were tested against males from the S-strain in 3 replications of each reciprocal. The mean reaction times were: S×N 251,29 sec., N×S 315,72 sec., S 215,56 sec. Thus throughout the resistance is lower than for females as is commonly found. The differences obtained were (N×S) — S 100,16 sec., (S×N) — S 35,73 sec., which gives (N×S) — (S×N) 64,44 sec. The difference between reciprocals is significant. A comparison with the results from the tests of females reveals that the male difference between reciprocals is greater, and the difference between (S×N) and S is less than for females. This indicates that the sensitivity is influenced by genes in the X-chromosome as the males from reciprocal crosses differ in respect of these and thereby acquire a genotype more consistent with their mothers than with their fathers.

In another experiment which included females of different ages the reciprocal F_1 offspring were compared with both the parental strains. All combinations of two between N, S, N×S and S×N, 6 in all, were tested together using females of 6 hr., 24 hr. and 60 hrs. age. Table 4 *a* gives the mean reaction times based on the 3 non-simultaneous tests and Table 4 *b* the differences between simultaneously tested groups. An analysis of variance (Table 5) indicated significant differences between

the two reciprocal F_1s and the two parental strains as well as between mean of parental strains and mean of F_1s. The mean differences between F_1 and the parental strains are, however, smaller than what was found in the other experiment and in particular the N×S-value for 24 hrs. is divergent. The possibility of confusion cannot be precluded here.

The age dependance was also investigated in a special experiment conducted in the same way as has been described for the pure strains. Simultaneous tests were made with females of the following types:

S×N 3 hr. — N×S 3 hr.; S×N 6 hr. — N×S 6 hr.
S×N 3 hr. — S×N 6 hr.; S×N 6 hr. — S×N 24 hr.
N×S 3 hr. — N×S 6 hr.; N×S 6 hr. — N×S 24 hr.
S×N 24 hr. — N×S 24 hr.

The mean reaction times are given in Table 6 *a*, and the differences between simultaneous tests in Table 6 *b*. The results indicate a constant resistance between 3 hr. and 6 hr. age but a considerable decrease between 6 hr. and 24 hr. The reaction to age can be said to be intermediate between the parental types in the beginning as the N-strain increases in resistance between 3 hr. and 6 hr. and the S-strain decreases, but after the age of 6 hrs. the decrease is more rapid in both F_1s than for any of the parental types.

TABLE 4 *a. Mean reaction times for females from parental strains and reciprocal F_1 offspring. Each value is a mean of 3 non-simultaneous tests.*

Age	Type			
	N	S	S×N	N×S
6 hr.	330,14	294,27	376,89	400,89
24 hr.	298,12	199,58	259,34	278,61
60 hr.	255,13	213,70	257,33	312,41

TABLE 4 *b. Differences between simultaneously tested groups. The significance of the differences is tested against a standard deviation of 17,62 sec. for differences between means of 19 animals; derived from the mean square within tests of 13,119 (see Table 5).*

Age	Difference					
	S×N − N	S×N − S	N×S − N	N×S − S	N×S − S×N	N − S
6 hr.	41,96*	88,00***	75,11***	108,95***	24,83	35,32*
24 hr.	−4,11	60,93**	−24,00	77,53***	40,23*	80,62***
60 hr.	−8,42	25,00	74,25***	73,62***	55,07**	45,98*

TABLE 5. *Analysis of variance of mean reaction times found in the experiment recorded in Table 4.*

Causes of variation	Degrees of freedom	Mean squares	Significances
Differences between ages	2	2919,404	
Differences between reciprocal crosses within ages	3	172,176	**
Differences between parental strains within ages	3	510,278	***
Differences between mean of parental strains and mean of F_1s within ages	3	646,233	***
Differences between repeated tests	24	24,958	**
Differences within tests	649	13,119	

TABLE 6 *a. Mean reaction times for reciprocal F_1 females of different age. Each value is the mean of 2 non-simultaneous tests.*

	3 hr.	6 hr.	24 hr.
N × S	429,32	428,72	338,50
S × N	389,08	397,50	296,90

TABLE 6 *b. Differences between simultaneously tested groups. The significance is tested against a standard deviation of 17,62 sec. (see Table 4 b).*

Differences between reciprocals		Differences between ages		
Age	N × S − S × N	N × S	S × N	Ages
3 hr.	21,14	3,33	−1,71	3 hr. — 6 hr.
6 hr.	32,00	73,43***	128,98***	6 hr. — 24 hr.
24 hr.	20,25			

Although there are discrepancies between the experiments as to mean reaction times they all give evidence of differences between reciprocal crosses. They also indicate an increase of resistance for both the reciprocal F_1s to a level higher than their respective mothers.

The offspring from the cross N × S are more resistant to the anaesthetic than the reciprocal cross. This difference cannot be attributed to chromosomal genes as both strains have free X-chromosomes (see chapter VIII) and the females thus attain identical genotypes, but must depend on some extra-nuclear factor, which is distributed in a different manner

TABLE 7. *Differences in mean reaction time for reciprocal F_1 females from crosses between the S-strain and various wild type strains.*

Wild type strain	Simultaneously tested			Tested against S					
	Number of test	Difference	Sign.	Number of test +×S	Diff. from S	Number of tests S×+	Diff. from S	+×S − S×+	Sign.
Algeria	14	—0,32	> 0,2						
Canton S	7	—8,11	> 0,2						
Florida	9	32,84	***	12	118,77	14	90,09	28,68	**
Karsnäs	4	24,75	> 0,2	2	76,08	5	15,99	60,09	*
Oregon	15	53,42	***	3	82,82	3	53,70	29,12	> 0,2
Stäket	2	—43,06	> 0,2						
Tunnelgatan	2	—3,02	> 0,2	2	45,54	2	38,38	7,16	> 0,2
Örebro	2	30,25	> 0,2	2	62,19	2	25,25	36,94	> 0,2

by egg and sperm. It is impossible to tell from an analysis of F_1 if the reciprocal differences depend on a maternal influence, analogous to the predetermination which causes reciprocal differences in the pigmentation of *Ephestia kühniella* (CASPARI, 1936) and the egg pigmentation of *Bombyx* (KIKKAWA, 1941, 1953). A predetermination in the proper sense is not likely in the present case, as such phenomena always have a tendency to disappear at some stage earlier or later during the ontogenesis.

It is important to know whether the difference between reciprocal F_1s is confined to crosses between the S- and the N-strain or if it is common to all crosses between the S-strain and other wild type strains. Data from other crosses are given in Table 7. Significant differences are found in the crosses to Oregon and Karsnäs, in the other cases the results are inconclusive. Most of these strains are, however, not specially inbred and it is possible that their genetical heterogeneity might have obscured the results.

MICHAELIS has studied the offspring from reciprocal crosses of 390 different strains of *Epilobium hirsutum* to the *hirsutum* strain Jena (MICHAELIS, 1947). In 42 % no reciprocal differences were found. The rest showed discrepancies of varying expressivity involving vegetative development. Other strains with special cytoplasmic features influenced the pollen fertility or flower development and caused varying differences between reciprocal crosses with respect to these characters (MICHAELIS, 1942; MICHAELIS and BAKKER, 1948). The conclusion drawn is that the cytoplasm determines the kind of reciprocal differences but the nuclear

genes determine their degree. It seems therefore likely that the genotypes of the different wild type strains in the present experiment may influence the degree of reciprocal differences in their reaction to anaesthetics.

V. BACK-CROSSES

Whether the difference between reciprocal F_1s is caused by some substance, synthesized under influence from genes of the mother and transmitted to her offspring by the cytoplasm of the eggs, or is caused by an autonomous cytoplasmic system can only be decided by further genetical analysis. CASPARI (1948) states: »Reciprocal differences in F_1 are certainly not sufficient, and their unchanged transmission to F_2 may be suggestive, but is certainly not convincing proof». If F_2 generations are raised from reciprocal F_1s the genes of the sex chromosomes will be unevenly distributed in the two F_2s, and may by themselves cause reciprocal differences.

Only after full substitution of the genome can the autonomy of a cytoplasmic system be proven. This has in the present investigation been accomplished in two ways. Firstly, the paternal genome has been introduced in the maternal cytoplasm by repeated back-crosses to males from the pure strain in question. In each generation of back-crosses the sensitivity has been tested. Secondly the chromosomes are substituted by outcrosses to a stock containing dominant chromosome markers and inversions to prevent crossing over (see chapter VIII).

The different lines of back-crosses and their designations are summarized in Table 8.

1. Back-crosses to S-strain

Line A. — Females from the offspring of S×N were back-crossed to males of the S-strain and in each succeeding generation cross-bred females were bred to S-males. The time of reaction was tested during 7 generations of back-crosses in 39 different tests with females of 24 hr. age. Table 9 and Fig. 5 give the mean differences from S in each generation. These values may be compared with an expected decrease from the F_1 value of the cross S×N which was 90 seconds. If a polygenic additively working system is assumed (see chapter VII) and effect of selection is neglected the decrease ought to be 50 % per generation as half of the chromosomes is substituted by pure S-genome in each cycle of reproduction. As the cross-bred animals are always the females, crossing

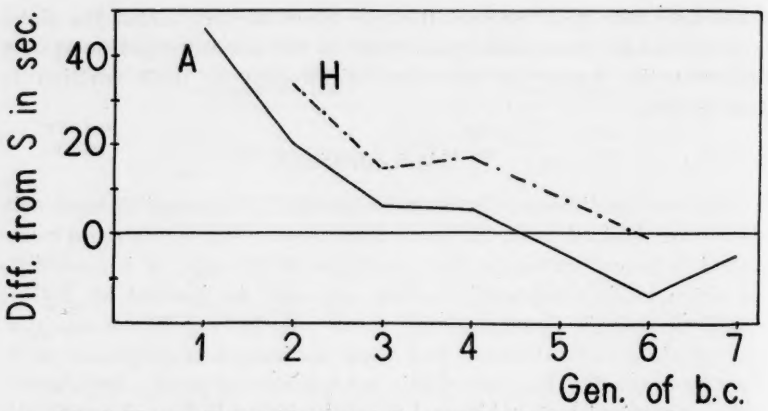


Fig. 5. The sensitivity of back-cross line A and series H from this line.

over must take place between the N- and S-chromosomes but this ought not to change the interpretations. The agreement between the experimental values and the expected is good and the differences from S disappear in a few generations. The results of this back-cross line can thus be quite satisfactorily explained as due to chromosome-carried polygenic differences between the N- and S-strain influencing the sensitivity to anaesthetics.

Line B. — This line corresponds to the A-line, but it originates from females of the other F_1, N×S, which are back-crossed to S-males. The restitution of the S-genotype ought to follow the same trend as in line A.

TABLE 8. *Outline and designation of back-cross lines. S/N offspring from S×N; N/S offspring from N×S. S....S repeated back-crosses to S; N....N repeated back-crosses to N.*

Crosses performed ♀	♂	Cytoplasm of the egg	Genotype to bee restituted	Designation
S/N	× S .... S	S	S	Line A
N/S	× S .... S	N	S	» B
S .... S	× S/N	S	S	» C
S .... S	× N/S	S	S	» D
S/N	× N .... N	S	N	» E
N/S	× N .... N	N	N	» G
S	× B or A	S	S	Series H
S	× H	S	S	» K
H	× S	S	S	» L

TABLE 9. *Reaction times for each tested generation of line A and series H from this line. Mean differences from simultaneously tested S-strain. Significances are calculated from a pooled mean square between repeated tests of 110,1. The expected values originate from the F_1 ($S \times N$) value of 90 sec., which is halved in each generation.*

Type		Back-cross generation No.					
		1	2	3	4	6	7
Line A	Mean diff. in sec.	46,5***	19,0***	6,7	6,4	—14,0**	—4,7
	Number of tests	6	6	6	9	6	6
Series H	Mean diff. in sec.		33,0***	14,5**	17,0**	— 0,6	
	Number of tests		6	6	9	6	
Expected		45,0	22,5	11,2	5,6	1,4	0,7

Mean excess of H over A, gen. 2—6 11,1***

25 back-cross generations were followed but only generations 1—11 and 19—25 were tested. The results for 24 hr. females are given in Table 10 and Fig. 6.

The time of reaction is much less in back-cross generation 1 than in the F_1, but thereafter the line stabilizes for many generations at a value significantly higher than S. Only very slowly does the mean reaction time approach the value of the S-strain, and in generations 19—25 taken together the difference is still significant.

There is thus a marked difference in behaviour of the lines A and B, and the problem to be solved is the cause of this difference.

One possibility is that the number of generations needed for restitution of the S-genotype is increased in line B due to some process of selection. The simplest explanation of such a selection would be attached X-chromosomes in the N-strain, as all females in the B-line then would get both their X-chromosomes from their mothers and thus originally

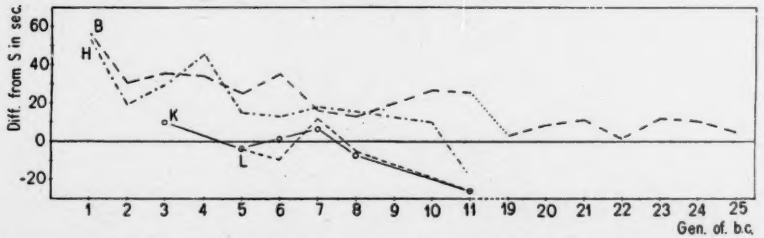


Fig. 6. The sensitivity of back-cross line B and series H, K and L from this line.

TABLE 10. *Reaction times for each tested generation of line B and series H, K, and L from this line. Mean differences from simultaneously tested S-strain. Significances are calculated from a pooled mean square between repeated tests of 252,7.*

Type		Back-cross generation No.																
		1	2	3	4	5	6	7	8	9	10	11	19	20	21	22	23	24	25
Line B	Mean difference in sec.	57,8***	31,2***	36,3***	34,0***	24,9**	35,7***	17,3*	12,8*	19,7	26,5	25,2	2,9	7,8	11,3	1,4	12,4	12,3	4,9
	Number of tests	3	4	3	4	5	5	4	6	1	1	1	6	11	6	6	2	4	4
Series H	Mean difference in sec.	57,4***	18,7*	28,9*	46,5***	20,3*	13,5	17,8			9,1	—19,4							
	Number of tests	2	3	2	2	4	5	3			3	1							
Series K	Mean difference in sec.			10,6		—3,1	0,8	7,5	—7,1			—27,0							
	Number of tests			2		4	5	1	2			1							
Series L	Mean difference in sec.						—8,7	12,9	—5,5			—26,4							
	Number of tests						5	1	2			1							

B 6—11 22,0***, B 19—25 7,0*
H 6—11 14,0**; K 6—11 —3,3 P 0,6—0,5; L 6—11 —7,6 P 0,2—0,1

from the N-strain. This possibility is, however, instantly ruled out by the fact that a cross between females of the N-strain and males possessing the dominant sex-linked gene Bar gave daughters all of which were heterozygous for Bar. Other possible chromosome abberations in the N-strain are made improbable by the fact that another wild type strain, Oregon, behaved in the same way as to differences between the A- and B-lines (see chapter VI). A general selection against the S-genome in the N-cytoplasm could also be suggested, but the existence of such a selection is not indicated in the substitutions by means of marked chromosomes, an account of which will be given in chapter VIII.

In addition the crosses constituting the series H, K, and L from the B-line throw some light on the question of delayed restitution of the S-genotype. They are also accounted for in Table 10 and Fig. 6. The series H consists of crosses between females from the S-strain and males from the back-cross line B. If the S-genome is not restituted in the B-line then N-genes will be transferred to the animals of the H-series and give them a value of reaction time higher than the pure S-strain. If, on the other hand, the higher resistance of line B is caused by some extra-nuclear factor transferred by eggs only, the animals of series H will be equivalent to pure S. In fact, the reaction times in the H-series are only a little below those of line B from corresponding generations and significantly above the time of the S-strain. However, if N-genes are postulated in the animals of the H-series, those cannot instantly disappear in another outcross to S. Such outcrosses are performed in the K-series where S-females are crossed to males from the H-series, and in the L-series where females from the H-series are crossed to S-males. None of these series differs significantly from S, but they are more sensitive than the flies in the B-line, which are outcrossed to S in a corresponding number of generations (*i. e.* those from the generation second to that from which males were taken to the H-series cross).

The H-series of crosses from the A-line also shows some peculiarity (Table 9 and Fig. 5). The time of reaction in the H-series turned out to be higher than in the corresponding generation of the A-line during the first generations. As the eggs in both cases transmit cytoplasm from the S-strain, and there ought to be no chromosomal differences, the higher resistance of the H-series is interpreted as an influence of the sperm from the cross-bred line that is greater than the influence from the eggs of the same line. This problem will be discussed further.

The results are best interpreted as a cytoplasmic difference between the A- and B-lines. In the B-line the cytoplasm from the N-strain is transmitted through the eggs, whereas this is the case for the S-cytoplasm in the A-line. The N-cytoplasm prevents the B-line from reaching the short reaction time of the S-strain, even when the S-genotype is fully restituted, and the active factor in the N-cytoplasm must therefore be autonomous. In some way the sperms from the B-line are also capable of transmitting this higher resistance almost unchanged when outcrossed to females of the pure S-strain (the H-series) but this higher resistance will not endure another outcross to pure S-animals (the K- and L-series).

The cytoplasm of the A-line, which originates from the S-strain does not change the genetically conditioned time of reaction, but when outcrossed to S-females the sperms of this line also show some capability to raise the reaction time to a higher level as long as the S-genome is not fully substituted.

2. Back-crosses to N-strain

Line E. — In this line F_1 females from the cross S×N were back-crossed to N-males. It was continued for 14 generations. Tests were performed with females of 24 hr. age in back-cross generations 1—11, and the results are given in Table 11. In no case were significant differences from the simultaneously tested N-animals found after the first few generations, where the N-level had not yet been reached. The experiment was repeated on a later occasion and was then followed for 5 generations of back-crosses. In this case the differences from the pure N-strain had already disappeared in the second generation.

Line G. — This is the back-cross line started from the reciprocal F_1 females, N×S. It was made simultaneously with the second E-line. In the chapter on the F_1 it was shown that the F_1 from the cross N×S had a reaction time much longer than that of the offspring from the reciprocal cross. In the first back-cross generation this difference has disappeared and from that generation on there is no different trend for line G and line E (Table 11). Both seem to stabilize at the same reaction time as pure N-flies when the influence of the S-genotype has been eliminated through the back-crosses to N-males. There is no indication of a delayed restitution of the N-genotype or of any influence of cytoplasmic factors.

These lines may, however, solve an important problem concerning the

TABLE 11. *Reaction times for tested generations of line E and line G. Mean differences from simultaneously tested N-strain. Significances are calculated from a pooled mean square between repeated tests of 251,7.*

Type		Back-cross generation No.					
		1	2	3	4	5	6–11
Line E	Mean diff. in sec.	—24,0	—29,3	16,5	—21,8	—43,8*	4,6
Exp. I	Number of tests	1	1	1	2	1	6
Line E	Mean diff. in sec.	—27,7**	2,0	0,1	—10,6	— 2,6	
Exp. II	Number of tests	3	5	4	2	2	
Line G	Mean diff. in sec.	—24,4*	— 8,5	0,6	18,4	—12,2	
	Number of tests	2	5	3	1	2	

E I 6—11 —4,6 P 0,5—0,4
E II 2— 5 —1,2 P 0,8—0,7
G 2— 5 —4,2 P 0,4—0,3

autonomy of the S-cytoplasm. If no autonomous cytoplasmic system is present in the S-strain then line E should be wholly identical to the N-strain when the N-genome has been restituted through the back-crosses. This was tested by reciprocal crosses between animals from back-cross generation 14 of line E and the S-strain, and by starting new back-crosses from these F_1s.

The first important question was: Would there be any reciprocal differences in F_1? 9 tests were made with $S \times E_{14}$ and S giving a difference of 90,2 seconds; 6 tests with $E_{14} \times S$ and S gave a difference of 104,4 seconds. The difference between the reciprocals were not significant and, further, their mean reaction times agreed well with that of the F_1 $S \times N$.

The back-cross lines to S were made in 4 different ways. They have been designated by the same letters that are given in Table 8 but are distinguished by a ′-sign following the letter, E_{14} corresponds to N. The A′- and B′-lines correspond to the A- and B-lines; in the C′- and D′-lines cross-bred males are instead back-crossed to S-females in every generation. These two lines were included to test whether crossing over by any chance influenced the rate of restitution of the S-genotype. However, all the lines followed the same trend as seen from Fig. 7 and Table 12, which agreed with the theoretical rate of substitution by back-crosses, and there was no indication that the cross-over chances in lines A′ and B′ made them behave differently from lines C′ and D′ where no crossing over could take place between the S- and the N-genomes.

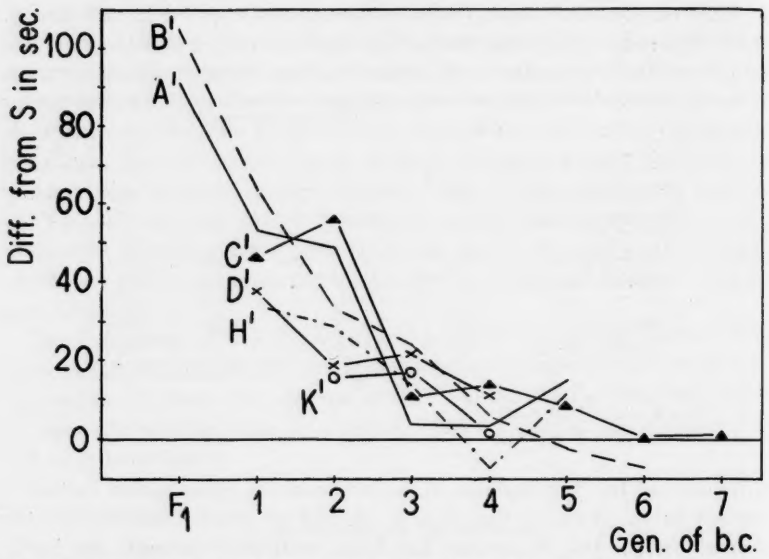


Fig. 7. The sensitivity of back-cross lines starting from the crosses N×E_{14} and E_{14}×N.

Crosses corresponding to the H- and K-series were also performed. If the differences between B′ and the S-strain are wholly due to chromosome-genetical differences, the rate of restitution must be greatest in K′ where the X-chromosomes are totally restituted without delay. The differences between all these series are, however, much too small and insignificant for any such evidence to be found. The time of reaction coincided with that of the A′-, B′-, C′-, and D′-lines at corresponding stages of back-crossing.

The interpretation of the results is as follows. As there are no reciprocal differences between the two F_1s and no different trend for the A′- and B′-lines, E_{14} cannot be identical to the N-strain. The theoretical probability that any of the S-genes remain in this line are very small (0,025 %) and even if assumed they could not explain the different behaviour of E_{14} and the N-strain in reciprocal crosses with the S-strain. Although the E-line cannot be phenotypically separated from the pure N-strain as to resistance to anaesthetics, there is no doubt that there is a remaining difference which is caused by the S-cytoplasm originally introduced by the S-egg in the cross S×N. It must therefore be assumed that the cytoplasm of E_{14} is different from the N-cytoplasm.

TABLE 12. *Reaction times for each tested generation of lines originating from the crosses between E_{14} and S. Mean differences from simultaneously tested S-strain. Significances are calculated from a pooled mean square between repeated tests of 462,0.*

Type		F_1	Back-cross generation No						
			1	2	3	4	5	6	7
Line A′	Mean difference in sec.	90,2***	54,0***	49,6***	3,0	2,7	15,8		
	Number of tests	9	6	3	6	2	2		
Line B′	Mean difference in sec.	104,4***	64,4***	33,0***	24,6***	7,2	— 3,7	—6,7	
	Number of tests	6	7	10	10	7	3	4	
Line C′	Mean difference in sec.		46,7***	55,8***	12,3	13,7	8,8	0,3	1,1
	Number of tests		6	11	7	9	7	4	4
Line D′	Mean difference in sec.		37,5	18,3	21,0	11,3			
	Number of tests		1	4	2	2			
Series H′ from B′	Mean difference in sec.		34,7	28,6**	14,2	— 7,5	11,8		
	Number of tests		1	4	2	1	2		
Series K′ from B′	Mean difference in sec.			15,3	16,2	1,4			
	Number of tests			4	2	1			

The S-cytoplasm in E_{14} prevents the stimulation that occurred when N-females were crossed to S-males and which thus must depend on an interaction between the N-*cytoplasm* and the S-sperm. Therefore no reciprocal differences were found in the F_1s between E_{14} and S. The mean reaction time of F_1 after the crosses $E_{14} \times S$ and $S \times E_{14}$ coincided with that of $S \times N$ which indicates that the N-genes can fully express themselves in the S-cytoplasm and that the sensitivity met with in these cases is the genetically conditioned one. Further, the remaining N-cytoplasm caused the different behaviour of the A- and B-lines. There is no difference between the A′- and B′-lines because E_{14} does not transmit any N-cytoplasm and A′ and B′ thus are identical both as to genes and cytoplasm.

The different behaviour of the lines B and B′ is a further proof of an autonomous cytoplasmic system in the N-strain as the cause of the remaining difference between line B and the S-strain. If this difference was caused by a delayed restitution of the S-genotype one should expect the same thing to happen in line B′, but in this line the S-genotype is restituted according to theoretical expectations. It will be shown in chapter VIII that the assumption of a selection for preservation of the N-genome in N-cytoplasm can be ruled out, and then the only possible explanation of the different behaviour of B and B′ is that the cytoplasm of line B originates from the N-strain whereas the cytoplasm of line B′ originates from the S-strain.

It is thus shown by the back-cross lines from the original F_1s and from E_{14} that both the N- and the S-strains possess some autonomous system; *i. e.*, a system capable of reproduction. An unsolved problem is, however, what causes the stimulation in the F_1 $N \times S$ and in line B. Does it occur if the sperm contains only S-genes or is the presence of S-cytoplasm also essential? This question can be answered by the outcrosses that were made from generation 19 of line B. The animals in this line after so many generations of back-crosses still showed a reaction time a little longer than the pure S-strain although the difference had decreased to about 6—7 seconds. As regards their chromosomes they must be identical to the S-strain, but their cytoplasm originated from the N-strain. The results of the reciprocal crosses to the N-strain and the back-crosses to S-males, lines A″ and B″, started from these F_1s are shown in Fig. 8 and Table 13. In none of the F_1s the reaction time is as high as in the F_1 from $N \times S$, but seems to agree with the other F_1; $S \times N$. Nor is there significant difference between the reciprocal F_1s.

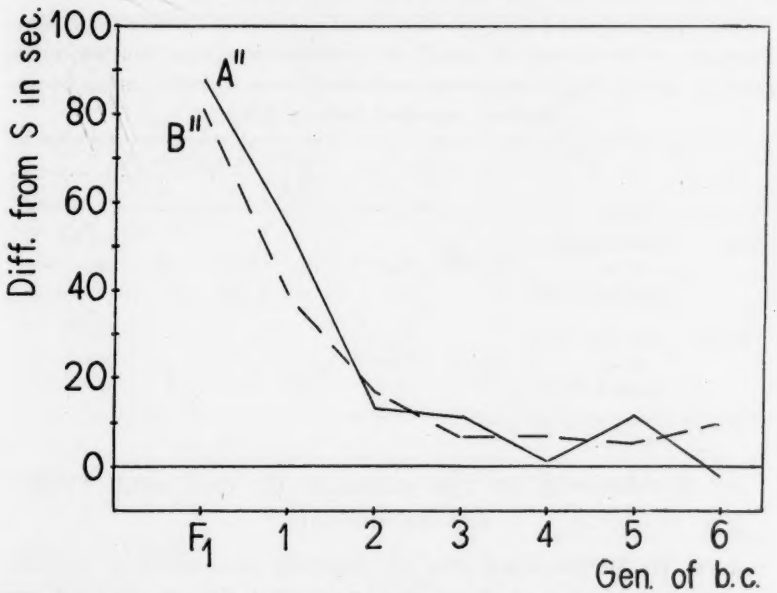


Fig. 8. The sensitivity of back-cross lines starting from the crosses N×B_{19} and B_{19}×N.

The two back-cross lines which correspond to lines A and B are parallel and rapidly decrease to the former level of the B-line.

No stimulation is thus accomplished by the cross N-female×B_{19}-male or the reciprocal. The S-genome alone is not enough as the cross N×S represents (N-genome + N-cytoplasm) × (S-genome + S-cytoplasm) and the cross N×B_{19} (N-genome+N-cytoplasm) × (S-genome+N-cytoplasm). In B_{19} the cytoplasm must thus still possess the characters of the N-strain although the animals phenotypically are very little divergent from the pure S-strain. The first back-crosses in lines B and B″ must be wholly homologous; they are both (N-+S-genome+N-cytoplasm) × (S-genome+S-cytoplasm). They also agree well in reaction times as most of the stimulation of the F_1 (N×S) has disappeared in B_1. But from there on the two lines follow different trends, as line B needs about 20 generations of back-crosses to S-males to accomplish the same decrease in reaction time as 3—4 generations in the B″-line. It is to be noted that the lines A″ and B″ are parallel and reach their lowest level at the same time but that both of them stabilize at a level more consistent with the B-line from which they originate than with the pure S-strain.

TABLE 13. *Reaction times for each tested generation of lines originating from the crosses between B_{10} and N. Mean differences from simultaneously tested S-strain. Significances are calculated from a pooled mean square between repeated tests of 325,3.*

Type		F_1	Back-cross generation No.					
			1	2	3	4	5	6
Line A″	Mean difference in sec.	88,2***	55,6***	13,3	11,4	0,1	11,3	—1,4
	Number of tests	4	3	6	5	5	5	3
Line B″	Mean difference in sec.	83,3***	39,5***	17,6*	8,6	8,0	6,1	10,7
	Number of tests	5	3	6	3	4	5	2

A″ 3—6 6,1***. B″ 3—6 7,9***

VI. OUTCROSSES OF THE S-STRAIN TO THE WILD TYPE STRAIN OREGON

It cannot be precluded that the observed phenomena in the outcrosses of the S-strain to the wild type of strain Florida, called N, are caused by a special nucleo-cytoplasmic system, characteristic exclusively for these two strains. The genetical analysis of the sensitivity was therefore supplemented by another analysis concerning crosses between the S- and the Oregon-strain, called Or. This wild type strain had been kept for a long time in the laboratory and was considerably inbred. As has been shown in chapter IV, Oregon was one of the strains that showed differences between reciprocal F_1s. The tests of mean reaction time was in this investigation always followed by weighing of the flies. The analysis was not followed for as many generations as the one involving the S- and N-strains, but 3—6 tests of each line were made in every generation.

The results are shown graphically in Figs. 9 and 10. The designations of the back-cross lines given in Table 8 have been used also in this case. The tests of the pure strains are recorded in Table 3, those of reciprocal F_1s in Table 7. In Fig. 9 only the tests made simultaneous to S are included. A comparison between the reaction times of the F_1s and the parental strains shows the same tendency as in the crosses between S and N, *i. e.* S×Or about the level of Or, Or×S > Or.

The trends of the A- and B-lines and of the H- and L-series from the B-line can also be seen to agree well with their homologues in the N-

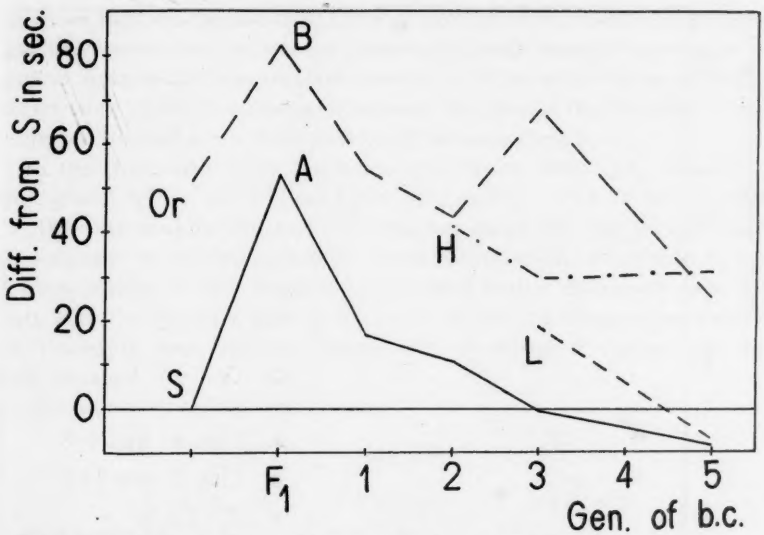


Fig. 9. The sensitivity of the wild type strain Oregon and its back-cross lines to the S-strain.

outcrosses. The A-line rapidly decreases to the level of the S-strain whereas the B-line lingers at higher values. The H-series from the B-line is only a little below the corresponding generations of line B, whereas the L-series very soon coincides with the pure S-strain. It can be mentioned that the excess of line B over corresponding H-generations is insignificant both for the N- and Or-outcrosses taken separately, but if both analyses are combined a significant value is obtained because the number of comparisons is increased.

The mean reaction times were compared to the mean weights of the flies in the pure strains, the reciprocal F_1s and the back-cross lines, as shown in Fig. 10. There was no correlation between weight and sensitivity, $r=0{,}65$ for 5 degrees of freedom. This is especially clear from the F_1s which are of identical weight but differ significantly in sensitivity. The size of the flies can thus be definitely ruled out as a cause of the differences in sensitivity.

VII. CHROMOSOME ASSAYS

MATHER has evolved a method for partitioning among the three major chromosomes polygenic differences between strains (MATHER, 1942;

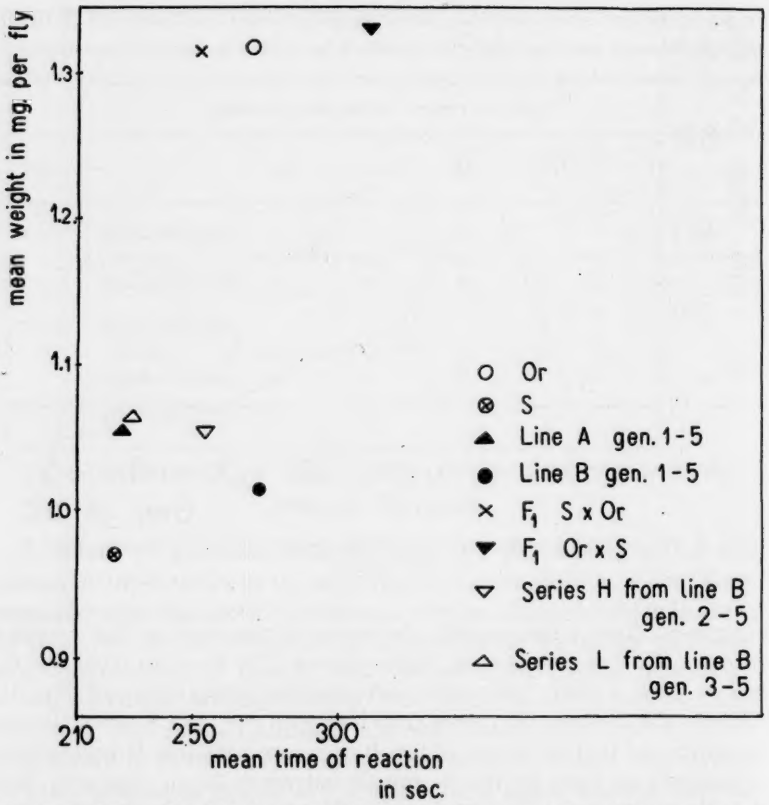


Fig. 10. Weight and reaction time for the strains Oregon and S, their reciprocal F_1s and back-cross lines to the S-strain.

MATHER and HARRISON, 1949). This method which he calls *chromosome assays*, is built on comparisons with a test-stock where all the major chromosomes are marked with dominant genes and which contains inversions which prevent crossing over and enable each chromosome to segregate as a unit. The effect of each chromosome is obtained as the difference between two chromosome combinations, one containing the marked test-stock chromosome, the other the unmarked chromosome from the strain that is tested. For each chromosome four equivalent differences are obtained and the assay value is calculated as the mean of these. There are certain weakenesses in the method as the influence

of genes that are dominant to those of the test-stock cannot be detected and as even the best test-stocks cannot absolutely prevent crossing over. But in spite of this the method turned out to be efficient in revealing differences in all chromosomes between the S- and the N-strain which were both tested as to their sensitivity to anaesthetics.

In the first experiment the marked inversion stock MI_1, containing the genes: y^{Si} sc^s InS y^{sP}; al^s Cy lt^s sp^s/dp b Pm^1; ru h D^s InCxF ca/Sb In(3R), was used as test-stock. The marker genes Pm and Sb were used to designate the second and third chromosomes of the test-stock. As the X-chromosome of this strain is not marked with a dominant gene but only with the recessive gene *y*, the origin of the X-chromosomes cannot be traced in the females, where only 4 different classes can be distinguished.

The following crosses were made:

	$S \times MI_1$	$MI_1 \times S$	$MI_1 \times N$
	↓	↓	↓
	$S/MI_1 \times S$	$MI_1/S \times S$	$MI_1/N \times N$
Back-cross No.	1	2	3

In the offspring from the back-crosses the following chromosome combinations segregated, and were collected and tested for mean reaction time in groups of ca. 20 animals.

Females	Males	
+	+	y
Pm	Pm	y Pm
Sb	Sb	y Sb
Pm Sb	Pm Sb	y Pm Sb

The mean times of reaction were recorded and used for the assays. Animals were collected and tested on three consecutive days. The rôle of the X-chromosome cannot be accounted for in the females, nor are the values for chromosomes II and III reliable as they contain the interaction term between these chromosomes and the X-chromosome. But in the males 8 classes can be distinguished and in this experiment, therefore, only male values were used. The mean reaction time in seconds was used in calculating the assays.

In the second experiment the test-stock used was a MI_2-stock where the X-chromosome of MI_1 had been replaced by a chromosome containing the genes sc^{S1} B InS sc^s with the dominant marker gene *Bar*. The following crosses were made:

	$S \times MI_2$		$MI_2 \times S$	
	$S/MI_2 \times S$	$S \times MI_2/S$	$MI_2/S \times S$	$S \times MI_2/S$
Back-cross No.	4	5	6	7

In these experiments only females were used in the tests. 8 chromosome combinations could be distinguished after the back-crosses:

+	B
Pm	B Pm
Sb	B Sb
Pm Sb	B Pm Sb

Groups of each kind were tested on two successive days, always simultaneously with a group of animals from the S-strain. In calculating the assays the differences from S were used in this experiment.

Back-crosses Nos. 5 and 7 do not account for the X-chromosome, as no females receive the X-chromosome from the test-stock.

In Table 14 the results of the chromosome assays are given for the X, II and III chromosomes. Their interactions were also calculated but throughout gave insignificant values. A special assay was calculated for each day, and thereby 3 independent estimates were arrived at in the first experiment, 2 in the second.

The different crosses between S and the test-stocks will differ in respect of their cytoplasmic content. Some of them will receive their cytoplasm from the S-strain, some from the test-stock. Further, in some cases the heterozygotes used are females, in some cases males, which may be of significance if crossing over is not completely suppressed. The assays are, however, calculated as differences between chromosome combinations originating from the same cross and thus it is not likely that these differences will influence the results. An analysis of variance performed on the values of Table 14 accordingly gave no significant differences between crosses that differed in these respects. A grand mean for each chromosome could therefore be calculated from all the S-values. They were compared with the means from the N-crosses. All chromosomes of the N-strain gave higher values than those of the S-strain, which except for the X-chromosome were also lower than the test-stock. The interaction terms were not significantly distinguished from zero but it was indicated that in the N-strain the effect of X together with the II and III chromosomes gave lower values than additiveness would suggest. As a whole the chromosome assays show

TABLE 14. *Chromosome assays for the S- and N-strains as to sensitivity to anaesthetics, recorded as mean reaction time. The significances of the differences between S and N are judged by a calculated mean square of 159,88 for differences between repeated tests giving standard deviations of 8,32 and 8,04 sec., respectively, for differences including 11 and 15 degrees of freedom.*

	Back-cross No.	X	II	III
Exp. 1				
$S/MI_1 \times S$	1	14,9	—21,1	14,5
		16,8	16,0	—18,2
		14,9	—14,3	— 4,8
$MI_1/S \times S$	2	18,3	— 4,5	—24,6
		22,6	—15,0	— 2,5
		—4,1	— 4,7	—12,1
Exp. 2.				
$S/MI_2 \times S$	4	19,5	—47,6	18,0
		18,2	—17,1	—11,2
$S \times MI_2/S$	5		16,1	12,2
			—33,9	15,7
$MI_2/S \times S$	6	21,7	—49,8	—11,1
		18,9	— 9,1	—10,8
$S \times MI_2/S$	7		—14,7	6,4
			—18,0	— 7,8
n		10	14	14
$\bar{x}$		16,17	—15,56	—2,59
$MI_1/N \times N$	3	51,0	17,5	10,5
		31,2	8,4	53,6
		17,3	2,3	0,6
n		3	3	3
$\bar{x}$		33,17	9,40	21,57
			Difference N — S	
		X	II	III
		17,00	24,96**	24,16**

that the differences in mean reaction time between the S- and the N-strain is at least partly dependent on chromosomal genes, and that these genes are distributed on all the major chromosomes. The genetical system influencing the sensitivity to anaesthetics can thus be said to be polygenic.

VIII. CYTOPLASMIC INTERCHANGE BY OUTCROSSES TO A MARKED INVERSION STOCK

The presence of a selection for preserving the N-genome in the N-cytoplasm cannot simply be ruled out. As the two pure strains are only distinguished morphologically by different numbers of sternopleural bristles which are guided by polygenic systems, a closer analysis must be founded on outcrosses to a third strain, possessing marker genes. Already the results of the crosses performed in the chromosome assays gave evidence that all chromosome combinations are viable, but no counts of their frequencies were made in that connection.

Another series of outcrosses to the stock MI_8 was therefore made, following the outline given below. The offspring from the last crosses were classified according to their marked chromosomes, and the number of flies of each combination was counted.

N-cytoplasm $\frac{N}{N}\frac{N}{N}\frac{N}{N} \times \frac{B}{\rightarrow}\frac{Cy}{Pm}\frac{D}{Sb}$

Control

N-cytoplasm $\frac{N}{B}\frac{N}{Cy}\frac{N}{Sb} \times \frac{S}{\rightarrow}\frac{S}{S}\frac{S}{S}$ S-cytoplasm $\frac{S}{S}\frac{S}{S}\frac{S}{S} \times \frac{B}{\rightarrow}\frac{Cy}{Pm}\frac{D}{Sb}$

(males from the S-strain)

N-cytoplasm $\frac{S}{B}\frac{S}{Cy}\frac{S}{Sb} \times \frac{S}{\rightarrow}\frac{S}{S}\frac{S}{S}$ S-cytoplasm $\frac{S}{B}\frac{S}{Cy}\frac{S}{Sb} \times \frac{S}{\rightarrow}\frac{S}{S}\frac{S}{S}$

8 different combinations 8 different combinations

It is evident from this outline that chromosome IV cannot be followed in these crosses. It is, however, very unlikely that the IVth chromosomes from the N-strain should not be substituted by such from the S-strain in back-cross line B. It is further excluded due to the system of propagation used for the pure strains that a mixture of different chromosomal configurations, free X, attached X, translocated and normal chromosomes, might have led to the results obtained in the back-cross lines.

In the analysis 25 females of the N-strain were crossed to males from the marked inversion-stock in pair cultures. All the F_1 females were heterozygous for the dominant gene *Bar* from the parental X-chromosome, thus giving an absolute proof that the X-chromosomes of the N-strain are not attached.

F_1 females containing the marker genes, *B*, *Cy*, *Sb*, were crossed to males from the S-strain, simultaneously with the first cross in the con-

TABLE 15. *Percentage of offspring from the outcrosses to the marked inversion stock MI_2 falling into different chromosomal classes.*

Chromosomal class			N-cytoplasm		S-cytolpasm (Control)	
			% Found	Deviation from exp. 12,5 %	% Found	Deviation from exp. 12,5 %
B	Cy	Sb	15,31	+2,81	16,14	+3,64
B	Cy		10,54	−1,96	10,00	−2,50
B		Sb	13,95	+1,45	13,86	+1,36
	Cy	Sb	15,65	+3,15	16,49	+3,99
B			11,56	−0,94	8,60	−3,90
	Cy		11,56	−0,94	10,18	−2,32
		Sb	11,56	−0,94	15,61	+3,11
	+		9,86	−2,64	9,12	−3,38
			n = 294		n = 570	
			$\chi^2 = 7{,}82$		$\chi^2 = 36{,}04^{***}$	
			P 0,5 — 0,3			
			Heterogeneity χ^2 4,56 P 0,8 — 0,7.			

trol series, the one between S-females and marked inversion males. The next crosses were also simultaneous in both series, and as regards chromosomes they were quite identical. Only the origin of the cytoplasm differed. The percentage of offspring that fell into the 8 chromosome classes is recorded in Table 15.

The deviations from expected percentages are significant for the controls but not for the flies with N-cytoplasm. In particular the classes containing the marker gene Sb seem to be overrepresented, which suggests that this chromosome contains genes which increase viability. The tendency is, however, the same in both cytoplasms and therefore is of no significance for the problem in question. The important thing is that there is no heterogeneity between the two series.

The results may be summarized thus: In both series all the 8 chromosomal classes segregate in fairly good agreement with theoretical expectations. Unless the marked inversion genome has changed the cytoplasm of the N-strain this proves that the N-strain does not favour other chromosomes at the expense of the S-genome, and thus that there cannot be any selection against this genome in the B-line. The analysis thus affords another indication that the restitution of the S-genome takes also place at an ordinary rate in the B-line and that its higher resistance to anaesthetics must be due directly to a cytoplasmic system

and not to a delayed restitution of the S-genome caused by a cytoplasmic system.

Among the 8 chromosomal classes obtained in the outcrosses, the one without marker chromosomes consisted of a pure S-genotype in N-cytoplasm. These animals afforded an opportunity for studying the interaction between genes and cytoplasm. For comparison the corresponding outcrosses were made to introduce the N-genotype into the S-cytoplasm:

$$\text{S-cytoplasm } \frac{S}{S}\,\frac{S}{S}\,\frac{S}{S} \times \frac{B}{-}\,\frac{Cy}{Pm}\,\frac{D}{Sb}$$

$$\downarrow$$

$$\text{S-cytoplasm } \frac{S}{B}\,\frac{S}{Cy}\,\frac{S}{Sb} \times \frac{N}{-}\,\frac{N}{N}\,\frac{N}{N} \quad \text{(males from the N-strain)}$$

$$\downarrow$$

$$\text{S-cytoplasm } \frac{N}{B}\,\frac{N}{Cy}\,\frac{N}{Sb} \times \frac{N}{-}\,\frac{N}{N}\,\frac{N}{N}$$

After this interchange of cytoplasm tests for reaction times were made, in which the cytoplasm-substituted flies were compared to the pure strains (Table 16 *a*). It appears to be both a gene dependent and a cytoplasmic difference between the strains. The gene-determined difference which appears from comparisons 4 and 5 is greater than the cytoplasmic one recorded in comparisons 2 and 3. This is also evident from comparison 6 where the two reciprocally cytoplasm-substituted strains are compared to each other. In fact, the influence of the S-cytoplasm on the performance of the N-genotype is very slight or absent.

Reciprocal crosses were also performed between strains with identical cytoplasms and different genomes and *vice versa*, Table 16 *b*. In comparisons 7 and 8 no differences were found between the reciprocal crosses which means that the S-genotype without corresponding cytoplasm (comp. 7) and the S-cytoplasm without corresponding genotype (comp. 8) are not able to produce any stimulation of the N-strain. Comparison 7 is to be compared to the crosses $N \times B_{10}$ and $B_{10} \times N$, which also were without stimulating effect. The difference found in comparison 9 shows that the N-cytoplasm is stimulated by the S-sperm even if it is not associated with the N-genotype. Comparison 10, finally, shows that a sperm containing N-cytoplasm but S-genotype, has a smaller but evident stimulating effect on the pure S-strain. This stimulation corresponds to the difference in reaction time between series H from line B and the pure S-strain.

TABLE 16. *Comparisons of mean reaction times for cytoplasm-substituted females and females from the pure strains. Differences between simultaneous tests. Significances are calculated from a pooled mean square between repeated tests of 472,25.*

N=N-genotype S=S-genotype //=N-cytoplasm ()=S-cytoplasm

(*a*) Influence of cytoplasm-substitution on pure strains.

Comparison No.	Constitution of flies		Difference I—II	Number of tests
	I	II		
1	/N/	(S)	119,5***	5
2	/N/	(N)	20,2	2
3	/S/	(S)	35,4*	2
4	/N/	/S/	72,3*	1
5	(N)	(S)	60,0*	1
6	(N)	/S/	17,0	1

(*b*) Crosses between cytoplasm-substituted and pure strains.

Comparison No.	Crosses performed		Difference I—II	Number of tests
	I	II		
7	/N/ × /S/	/S/ × /N/	11,5	1
8	/N/ × (N)	(N) × /N/	0,0	1
9	/S/ × (S)	(S) × /S/	30,2***	9
10	(S) × /S/	(S) × (S)	17,0	3

IX. INVESTIGATIONS CONCERNING THE MEAN EFFECTIVE DOSE (ED50)

In all experiments hitherto the mean reaction time has been used as the criterion of sensitivity, and the interpretations are thus built on observations of different rates of reaction. With the concentration of anaesthetics used in these experiments it takes only about 10 seconds until the flies are brought to immobility. The gaseous absorption through the cuticula can then hardly be of importance and the transport into the central nervous system must take place mainly *via* the tracheae.

In *Drosophila* the tracheal system is connected to the exterior through the tracheal openings, the spiracles, 2 pairs on thorax and 7 pairs on the abdomen. On the head they are absent. The tracheae ramify into

the whole body and in some places they dilate into thin-walled air sacks, which presumably facilitate the gaseous exchange between the respiratory system and the tissues. In the head and thorax these air sacks are particularly well developed. As the tracheae ramify into the tissues their diameters decrease but whether the terminal branches penetrate the cellular cytoplasm is still a matter of dispute (EDWARDS, 1953).

The central nervous system in the head, the cephalic mass, consists of the supra- and the sub-oesophagial ganglia, compactly merged together around the oesophagus and connected to the thoracic mass by the cervical connective. The thoracic mass is a fusion product of the thoracic and abdominal ganglia. No ganglia occur in the abdomen which is innervated by nerve trunks from the posterior part of the thoracic nerve centre (MILLER, 1950).

In chapter II it was noted that the difference in mean reaction time between the S- and the N-strain persisted both if the flies were brought to immobility by a small dose of anaesthetics or were killed by a higher dose. As far as only the time of reaction is concerned, this difference may depend on anatomical or morphological differences that influence the gaseous exchange of the tissues.

However, if the flies are subjected to very low doses of anaesthetics during a long period there is a certain threshold concentration below which the animals remain apparently unaffected. Presumably too few reactive groups are inactivated by the anaesthetic, and the number of affected groups must have attained an equilibrium with the concentration in their environment. This concentration need not be the same as that of the atmosphere as it is possible that there exist several equilibria between the air and the reactive groups in the cytoplasm. If this threshold concentration is different for the two strains it is not likely that the gaseous exchange is deciding their sensitivity, and the difference must then be a quantitative or qualitative difference in the sensitive intracellular system. Of course, other things may also influence the threshold value, for instance the fat deposite or amount of different lipids, but such characters are presumably gene dependent and ought to be compensated in the reciprocal F_1s.

The mean effective dose, ED50, is the concentration that affects 50 % of the treated animals and leaves the other 50 % unaffected, and this measure thus affords an estimate of the threshold value sought for. If ED50 is different for the S- and the N-strain and for the reciprocal F_1s then the differences must be due to intracellular factors.

For determination of ED50, 6 of the glass tubes used for the tests of

mean reaction time were calibrated to identical volume by moving the rubber stoppers. Tests were made for a suitable dose range, and each tube was then supplied with a certain amount of ether so the concentrations formed an increasing series with the dose of the last tube about twice that of the first. The test groups consisted of 10 or 15 animals. Males and females were tested simultaneously in the two halves of the tubes. They were allowed to remain in the tubes for 2 hours. After 1 hr. and at the end of the experiment the number of anaesthetized and unaffected flies were recorded. The results from 1 hr. and 2 hr. treatment were very similar and in the calculations of ED50 only the 2 hr.-values have been used. The temperature during the experiments was kept at 20° C.

ED50 for immobility was determined instead of LD50 (mean lethal dose) as complications interfered with the classification into living and dead. Flies that had been deeply anaesthetized for as long as 2 hr. seemed to have suffered irreversible damage. When left to recover in ether free atmosphere they would be motionless during many hours showing only some slight muscular spasms and would then usually die without ever having recovered to normal activity. Also when ED50 for immobility was determined some cases of irreversible damage were recorded but these did not interfere with the classification.

The percentages of affected animals were transformed to probits and plotted against the dose on logarithmic-probit graph (MILLER and TAINTER, 1944). A straight line was fitted to the points by eye and the concentrations corresponding to the probit value 5 was read off. It was not found necessary to calculate the probit line arithmetically as the differences between repeated experiments, caused by variations in the doses, were greater than the error that could be caused by wrong fitting of the probit line. The ED50 values were compared in an analysis of variance (Table 17).

ED50 was determined for N- and S-males and females of different ages, from 3—96 hrs., and for the offspring from reciprocal crosses between the two strains. The results are given in Table 17. The pure strains show the highest resistance at 3—6 hrs. of age, then stabilize on a somewhat lower value. The males are throughout more sensitive than the females. Most important, however, is that the values for the S-strain for all ages are lower than for the N-strain.

The reciprocal F_1 animals were tested at 4 different ages, from 24—136 hrs. The value for S×N 24 hrs., is unexpectedly low, but generally the results are consistent and the values for N×S are significantly higher

TABLE 17. *ED50 for N-, S-, and reciprocal F_1 flies of different ages. The values refer to the volumes of ether injected in the testing tubes, measured in ml. 10^{-3}. The corresponding percentage of ether in the gas were calculated to 1,30 % for 0,030 ml.*

Pure strains.

Age in hours	N ♀♀	S ♀♀	N ♂♂	S ♂♂
3	26,5	25,5	24,0	23,0
3,5	29,0	27,5	25,5	25,0
4	30,5	29,5	26,5	25,5
4,5	29,5	29,0	27,5	26,0
6	29,5	25,5	23,5	23,0
12	28,5	26,0	25,5	23,0
24	28,5	24,5	23,5	22,0
60—96	25,0	22,5	21,5	18,5

F_1.

Age in hours	N×S ♀♀	S×N ♀♀	N×S ♂♂	S×N ♂♂
24	27,0	22,5	23,0	19,0
36	27,5	25,0	25,0	23,0
72	27,5	25,5	—	—
136	27,5	26,5	25,5	24,0

Analysis of variance:

Pure strains.

Source of variation	Degrees of freedom	Mean square	Variance ratio and probability
Between different ages	7	34,23	3,10*
Between sexes within ages	8	23,72	5,04**
Between N and S within ages and sexes	16	4,71	2,84**
Between repeated tests	28	1,66	

F_1

Mean square for variation between reciprocal F_1s within ages and sexes: 3,84

Degrees of freedom: 7.

Variance ratio $\frac{3,84}{1,66} = 2,31$*.

than for S×N. For corresponding ages the values for N×S are higher than those obtained for N-flies and the values for S×N higher than for S-flies, except at 24 hrs. age. The variation between experiments is, however, too great to allow any judgement of the significance of this comparison.

Generally, however, it can be said that the values for ED50 are in agreement with those for mean time of reaction. It is fair to suppose that the results of the back-crosses would have been the same, had ED50 been determined instead of mean time of reaction, but as the time experiments are much easier to carry out they have been preferred. The consistency between dose and reaction time is important, for it suggests that the differences in reaction time are also manifestations of the intracellular system which reacts with the anaesthetic. The nature of this system will be further discussed. It must be looked upon as an active system that is inactivated by the anaesthetics.

X. DIFFERENT THEORIES CONCERNING THE MODE OF ACTION OF ANAESTHETICS

The hereditary analysis indicates that the differences in sensitivity depend both on a genetical and a cytoplasmic system. The determinations of ED50 further indicates that the differences must be sought for in some intracellular system. At this point it is therefore necessary to look further into the current theories about the mode of action of anaesthetics.

As the number of substances that may act as anaesthetics is increased, the difficulties of finding any consistency between these substances have grown, and it has become clear that there is no specific structure necessary for anaesthetic activity.

The absence of any characteristic atomic configuration necessary for anaesthetic activity has directed attention towards the physical properties that may be common to anaesthetics and that may explain quantitative differences in activity. Many investigators have tried to find correlations between anaesthetic activity and physical qualities within homologous series of substances. Already some 50 years ago OVERTON and MEYER found a positive correlation between the anaesthetic activity and the oil/water distribution coefficient for some groups of compounds. The lipid-solubility rule which they founded does, however, not tell anything about the manner of action of anaesthetics but only about an observed correlation, and has not been found to be generally applicable.

The same is true for the correlation found by TRAUBE (1904) between inhibiting activity and ability to depress the surface tension of water. According to this theory, the main property of anaesthetics should be their ability to change the permeability of the cells by accumulation in some cell surfaces.

As the cell membrane is supposed to play an important rôle in the conduction of excitations, there is reason to believe that an anaesthetic substance which inhibits such excitability, should exert its effect on such a membrane. The theory of lipid-solubility has also drawn attention to the cell membrane. LILLIE (1923) supposed that through the inhibiting effect of anaesthetics the increase in permeability that presumably gives rise to the depolarization accompanying a wave of excitation, fails to appear. His theory would only be valid for the increase of permeability that accompanies an excitation. BUTLER (1950), in his review, states »that it would appear that excitability of nervous tissue can actually be abolished by an alteration of the properties of the membrane in a manner similar to that suggested by LILLIE», but DRILL (1954) supposes that if an alteration of permeability really takes place it presumably is a secondary consequence of a direct metabolic change caused by the anaesthetic. It is not at present possible to tell if anaesthetics have such a definite effect on permeability that it would explain its mode of action.

Several anaesthetics are known to inhibit certain oxidative reactions in the brain, and especially QUASTEL and collaborators have supposed that the observed inhibition of oxygen consumption is a direct effect of the anaesthetic on some enzyme. The process of removing hydrogen from a substrate to molecular oxygen is a complicated series of reactions, and all the oxidative processes are not inhibited to the same degree by anaesthetics. It has been shown that the most anaesthetic-sensitive part of the chain is the one between cozymase and cytochrome c, according to the following survey. As yet very little is known of these processes.

Substrate-Dehydrogenase-Cozymase-Flavoprotein-Cytochrome-Cytochrome Oxidase-Oxygen
Anaesthetic insensitive region | Anaesthetic-sensitive region | Anaesthetic insensitive region

The inhibiting effects of the anaesthetic substances are reversible (QUASTEL and WHEATLEY, 1934) but high concentrations produce irreversible changes. According to QUASTEL, a rapid equilibrium is attained between the anaesthetic and a constituent of the respiratory system, but there is also a slow development of irreversible changes leading to increased inhibition of respiration, that cannot be restored

to normal by removal of the anaesthetic. He supposes that this depends on a gradual irreversible denaturation of the enzymes. It may be compared with the observation during the ED50 determinations in the present investigation, that an irreversible change occurred when the flies were exposed to the anaesthetics for a long time.

Those parts of the oxidative hydrogen transport that are supposed to be anaesthetic-sensitive are also essential for the production of energy-rich phosphorus compounds and for the synthesis of acetylcholine. For the maintenance of nervous activity a reserve of energy-rich phosphorus compounds is presumably of vital importance. If the oxidative metabolism is inhibited, the synthesis of acetylcholine is depressed. The inhibited synthesis of acetylcholine may cause the inhibited nervous function during anaesthesia because the metabolism of acetylcholine presumably controls the permeability for ions and metabolites in nervous tissues. This theory facilitates the assumption of a common centre of action for a great number of anaesthetics. It is evident that some intracellular enzyme system is inhibited in its normal function by the anaesthetics but if this inhibition affects some specific link of the oxidative metabolism or some enzyme system which connects metabolism to nerve function cannot be decided as long as our knowledge about the casual connection between metabolism and the function of the nerve cells is so incomplete.

XI. EXPERIMENTS CONCERNING THE RESPIRATION INTENSITY

In the present investigation it was possible to establish differences in sensitivity to anaesthetics between the S-strain and other strains. If these differences are caused by a quantitative difference in the activity of some anaesthetic-sensitive enzyme system, it might be possible to prove this by measuring the respiratory activity *in vivo* of the strains, without addition of anaesthetics. In such an experiment it is impossible to find the intensity of the central nervous system exclusively, but total respiration intensity has to be measured. If differences are found it is, however, more probable that they indicate metabolic differences in the central nervous system than that they should be limited to other organs.

Experiments were made to determine the respiratory intensity of the flies using an apparatus that in principle agrees with a method described by GERARD and HARTLINE (1934). Long narrow pipettes, holding 0,1 ml. and graded to 0,01 ml. were used. All the pipettes used had the same diameter, the intervals between scale lines thus agreed. Flies of

TABLE 18. *Results from determinations of respiration intensity for females from pure strains and reciprocal F_1s.*

Mean change of gaseous volume/fly during 2 hours in mm³:

Type of flies	20° Number of flies/exper.		25° Number of flies/exper.		30° Number of flies
	1	15	1	5	15
N	2,92	3,05	4,29	3,68	4,71
S	2,89	2,90	3,99	3,68	4,55
N × S	2,89	3,56	4,84	3,54	—
S × N	3,50	3,57	5,03	3,95	—

Analysis of variance:

Source of variation	Degrees of freedom	Mean square	Variance ratio	Probability
Between different temperatures	2	39,232	4,03	0,2
» » number of flies/exp. within temperatures	2	9,725	9,17	**
Between different types of flies within temp. and equal-numbered groups	13	1,060	1,66	0,2—0,05
Between S and N	5	0,125	—	
» reciprocal F_1s	4	0,468	—	
» pure strains and F_1s	4	2,822	4,41	**
» experiments with the same kind of flies	150	0,640		
Between different number of flies/exp.	2	15,195	—	
» » temperatures within equal-numbered groups	2	33,762	31,85	***
Between different types of flies within temp. and equal-numbered groups	13	1,060		

known age and sex were placed in short narrow glass tubes and were forced together by cotton stoppers from both sides, so tight that their mobility was completely inhibited. The glass tube was connected to a pipette by a short piece of rubber tubing and within the pipette a known amount of 20 % NaOH was injected at a certain place. The open end of the glass tube containing the flies was closed by a rubber stopper. When CO_2 is formed by the respiration it is dissolved in the NaOH-

solution and the gaseous volume is accordingly decreased as more and more of the oxygen is replaced by CO_2. The distance that the column of liquid has moved is thus a measure of the intensity of respiration. The apparatus was kept horizontal in an incubator at 20° C and the first reading was made after 15 minutes when temperature balance was supposed to have been attained. Further readings were made 1 and 2 hours later. If the decrease during the first and second hour is the same this is a proof that no lack of oxygen can have influenced the results. In one experiment readings were made up to 4 hours from the start, but no decrease of respiration intensity was found. For the statistical computations the 2 hr.-values were used. The experiments were made with varying number of flies and at 3 different temperatures. The pure strains S and N and the reciprocal F_1s between them were tested. The results are shown in Table 18. From the analysis of variance it is clear that the intensity is influenced by as well temperature as number of flies per experiment. An increase of temperature induces a higher respiration intensity. Tests of sensitivity have also been made at different temperatures and it was then found that the mean reaction time decreased with increasing temperature. The differences between the N- and S-strain and between reciprocal F_1s, however, remained unchanged at all tested temperatures. The respiration experiments also reveal a higher intensity for the F_1s than for the pure strains; presumably a manifestation of hybrid vigour, but do not indicate any differences in intensity either between the pure strains or between reciprocal F_1s. This seems to rule out the assumption of quantitative differences in the oxidative metabolism, and if the anaesthetics at all interfere with these processes, the divergent behaviour of the S-strain must be caused by a qualitative change in some enzyme structure, which can only be observed by exposure to anaesthetics.

XII. ADDITIONAL EXPERIMENTS WITH THE PURE STRAINS

1. Repeated anaesthesia

Some experiments were performed to investigate whether the deviations from the mean reaction time in each test were purely accidental or whether they depended on heterogeneity of the material, for instance, in size and activity. If a presumably homogeneous collection of flies were to be divided into smaller batches according to the sensitivity of the flies and if these batches were tested separately on a later occasion no correlation would be expected between the rank order of the batches

at the first and the second test, if the deviations were random. If, however, some biological diversity caused the differences in sensitivity in the first test, it would also assert itself on the second occasion, and the rank orders would be correlated.

To test this, 100—200 females 48 hours old were anaesthetized in a separation funnel measuring 440 ml. In the tap of the funnel was a space for measuring off quantities of 1 ml. When the anaesthetic had begun to work the tap was turned half round and the immobilized flies fell down in the space, whereas the unaffected flies climbed to the walls of the funnel. The tap was turned another half round and the immobilized flies were let out and collected in a vial. The procedure was repeated every 15 seconds and the flies were divided into batches with increasing resistance. The letting out of the flies through a lock did not appreciably change the ether concentration during the test. After 2—6 hours each batch was tested separately for mean reaction time in the usual way.

Tests were performed with flies both from the S- and N-strains. The correlation coefficients were for S $0{,}_{082}$, 6 degrees of freedom, and for N. $0{,}_{551}$, 8 degrees of freedom, both positive but insignificant. The homogeneity of the strains is thus reliable. The slight indication of a positive correlation was found to depend on another phenomenon. It was observed during these experiments that if the same dose was used in both tests the mean time of reaction was always higher in the second test than in the first. The first etherization had increased the resistance of the flies. This phenomenon was further analysed by using 5 different related anaesthetic substances, *viz.* diethylether, ethylvinyl ether, divinyl ether, furan, and tetrahydrofuran. In the first place doses giving about equivalent mean times of reaction to the different substances were determined. To a dose of 0,1 ml. diethyl ether corresponded $0{,}_{05}$ ml. ethylvinyl ether, $0{,}_{06}$ ml. vinyl ether and 0,1 ml. furan. Then in the first experiment a large sample of 60 hr. old females was anaesthetized and the mean reaction time determined on a random sample of 20 animals. The flies were divided into 6 batches each of which was tested a second time after varying intervals of time from $0{,}_5$ to 32 hours. Tetrahydrofuran seemed to induce an irreversible change in the central nervous system as the flies never recovered from the first anaesthesia. The results for the other substances are recorded in Fig. 11. An increased resistance is found both for the S- and N-strain when diethyl ether and ethylvinyl ether are used as anaesthetics. The increase which is highest for ethylvinyl ether reaches a maximum after $3{,}_5$—$7{,}_5$ hours then slowly decreases. Divinyl ether and

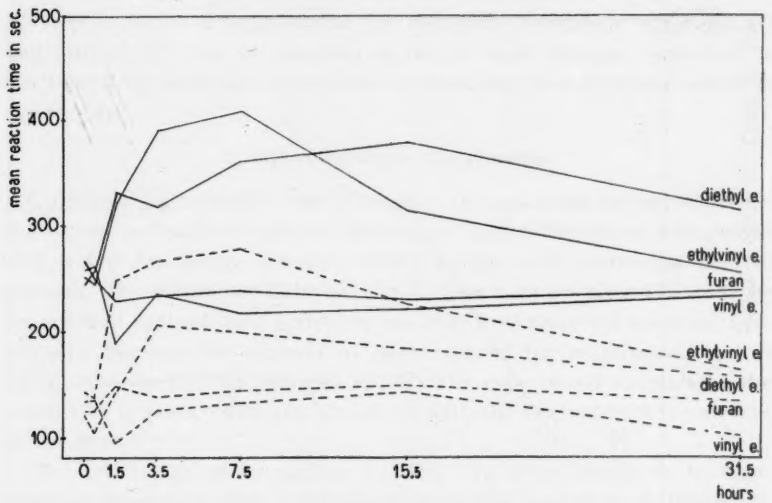


Fig. 11. Effect of a preceding anaesthesia on the sensitivity at a second test, performed at different time-intervals from the first treatment. ——— the N-strain, — — — the S-strain.

furan, on the other hand, did not induce any increased resistance, as the mean reaction times were about equal during the whole experiment. Other tests where divinyl ether was used for the first anaesthesia and diethyl ether for the second did not indicate any increased resistance. As a consequence of this, divinyl ether was always used when the flies were distributed into vials before the tests of reaction time (see ch. II). An induced increase of resistance might otherwise obscure the results.

A second experiment concerning the increase in resistance was made, where the flies were anaesthetized repeatedly with intervals of about 1 hr. 60 hours old N- and S-females were treated with diethyl and ethylvinyl ether. Both strains reached levels of resistance much higher than normally, the S-strain passing even the normal level of the N-flies (Fig. 12). At 5,5 hr. the diethyl ether seems to have brought the N-flies almost to the threshold above which the flies cannot be immobilized by the dose in question, for some flies were still unaffected after more than 10 minutes. Otherwise the behaviour of the two strains is almost parallel, the S-strain reaching its maximum level possibly a little sooner than the N-strain.

Another question arises. Does the increase in resistance depend on a

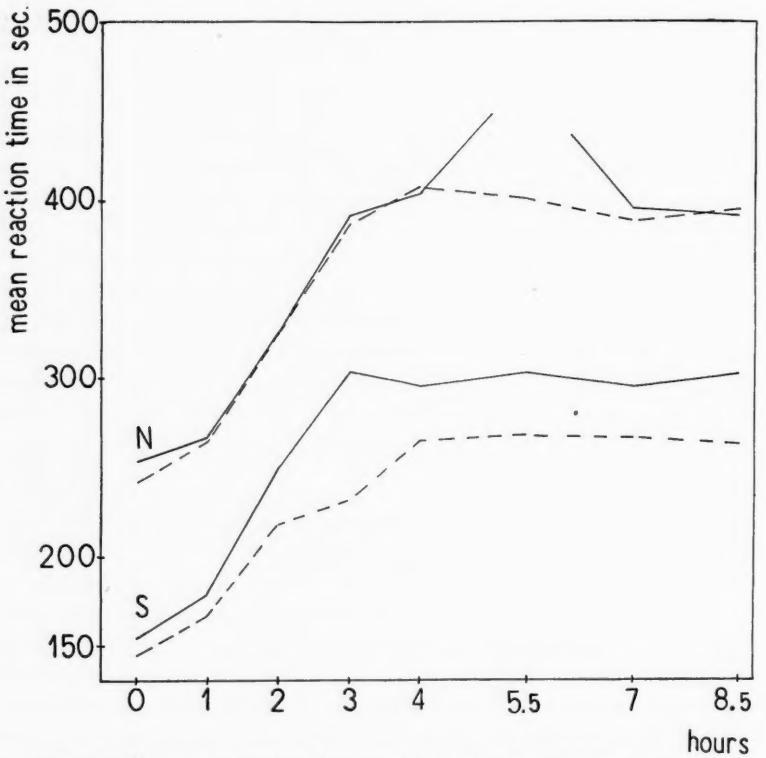


Fig. 12. Effect on sensitivity of repeated anaesthesia with 1 hr. intervals. ——— diethyl ether, — — — ethylvinyl ether.

change in reaction time or has the concentration needed for anaesthesia increased? This question can be answered by determination of ED50 for repeatedly anaesthetized flies and flies that had not been treated with diethyl ether before. Such tests were made with S- and N-females about 60 hrs. old. Each strain was divided into two batches, one of the batches being anaesthetized hourly five times, the last time being one hour before the ED50 test; the other batch being tested for ED50 without previous treatment with diethyl ether. The ED50 values found coincided exactly for treated and control animals in both strains. The increase of resistance caused by repeated anaesthesia must depend on a retarded inactivation of the anaesthetic-sensitive receptors in the central nervous system, and not on a higher reaction threshold. The ability to

develop a retarded inactivation by repeated treatments with diethyl and ethylvinyl ether is common to the N- and S-strains and thus is not bound up with the system that determines their different reaction thresholds.

2. Miscellaneous experiments

To investigate whether the sensitivity is associated exclusively with the supra- and sub-oesophagial ganglia, N- and S-flies were decapitated and tested for mean reaction time together with untreated control animals. The difference between the N- and S-strain persisted, but the decapitated animals had a shorter reaction time than the controls, presumably because the amount of ether needed for inactivation is less when only the thoracic ganglia is left. The experiment shows that the sensitivity is not exclusively bound up with the central nervous system of the brain.

The activity of extra-nuclear systems are often found to be temperature-dependent. This is the case with the CO_2-sensitivity (L'HÉRITIER, 1948) and the »sex ratio» factor of *Dr. bifasciata* (MAGNI, 1953). Tests were performed to investigate whether the sensitivity to ether was influenced by temperature treatments. Pupae of both strains were allowed to develop at 32° C and the flies were tested at 24 hr. of age. Young imagos were submitted to temperature shocks of varying duration at 32° and 35° C and were then tested for reaction time. None of these treatments had any influence on the sensitivity either of S- or N-animals.

It has already been stated that the sensitivity of the S-strain asserted itself also for ethylvinyl ether, divinyl ether and furan. Some other substances have also been tested in less detailed experiments. Differences in sensitivity were found for CO_2, but both strains recovered from the anaesthesia, thus no system of the kind studied by L'HÉRITIER and TEISSIER is involved. Some organophosphorus compounds of the anticholinesterase type and urethan also affected the S-strain more rapidly than the N-strain. Urethan had to be given to the flies in the food, a rather unreliable method, but differences in reaction time could still be observed.

Attempts were made to transplant haemolymph between S- and N-animals and between offspring from reciprocal F_1s according to the method described by L'HÉRITIER and SCOEUX (1947). Control animals were injected with their own kind of haemolymph. The animals carrying transplanted haemolymph were tested for reaction time each day as

long as they could be kept alive. No consistent effects of the transplantations could be observed. Assuming that the number of animals, 10—15, was sufficient material for a reliable judgement, the negative result indicates that the difference between the strains is not caused by an infective system. This distinguishes it from the CO_2-sensitive system (L'HÉRITIER and SCOEUX, 1947) which is transplantable and depends on a qualitative difference.

XIII. DISCUSSION

If the anomaly in the S-strain which causes its outstanding sensitivity to anaesthetics were exclusively gene dependent, the reaction time in the back-crosses would oscillate between the N- and the S-level following the restitution of the genome in question. If, on the other hand, it were caused by an autonomously working cytoplasmic system, without the influence of the chromosomal genes, the character of the genome would be negligible and the sensitivity of the flies would be completely determined by the origin of their cytoplasm. It is evident from the back-crosses and the cytoplasm-substitutions that none of these alternatives alone can explain the hereditary mechanism of the sensitivity.

In reality no absolutely certain case of cytoplasmic autonomy is known. When the analysis is sufficiently penetrating a gene dependance always seems to be involved. The biochemical analysis of vegetative littles in the American strain Yeast Foam (EPHRUSSI and collaborators, 1949 a, b, c, 1950) indicated that the cytoplasmic mutation present in this strain was autonomous both as to reproduction and function. On a certain French yeast strain the analysis could be carried further and then revealed a nucleo-cytoplasmic interaction (CHEN, EPHRUSSI, HOTTINGUER, 1950). The CO_2-sensitivity in *Drosophila melanogaster* found by L'HÉRITIER and TEISSIER (1937) also seemed to depend on an autonomous cytoplasmic factor. This factor can in suitable genetic environments exist in several mutant forms and is not only transmitted by the cytoplasm from mother to offspring but also by aid of organ transplantations and haemolymph injections. However, GUILLEMAIN and PLUS (1953) could also in this case establish the existence of a gene, capable of preventing reproduction of this factor.

The gene dependance of the sensitivity is clearly manifested in the cytoplasm-substitutions and in the back-cross line A where the S-genotype is restituted after the cross S×N. Nor do the back-cross lines from E_{14}, A′, B′, C′, and D′ suggest anything other than a substitution of the N-gentoype by an S-genotype according to theoretical expectations.

It is essentially the cross N×S and the back-cross line B, which restitutes the S-genotype after this cross, that suggest some other influence. The evidence that the rate of restitution of the S-genotype is the same in this as in line A is considerable. The outcrosses to the marked-inversion stock and the parallel behaviour of the Oregon-strain do not indicate any chromosomal aberrations or any selection against the S-genotype in N-cytoplasm. The rate of restitution of the lines B′ and B″, which as regards the genomes are identical to line B, does not deviate from the normal rate (represented by line A), nor do the reaction times of series K and L from line B exceed the S-level.

The influence which causes the cross N×S to exceed S×N in reaction time, and which prevents line B from reaching the sensitivity of the S-strain for more than 25 generations must thus be regarded as cytoplasmic. The cytoplasmic origin of the female parent is the only factor which differentiates the crosses N×S and E_{14}×S, and the cytoplasmic origin of the male parent the only factor which differentiates N×S and N×B_{19}. N×S has, however, a mean reaction time about 117 seconds longer than that of the S-strain whereas E_{14}×S and N×B_{19} exceed the S-level with 104 and 83 seconds, respectively. The different trends for the back-cross lines starting from these crosses, line B on one side and lines B′ and B″ on the other, are even more striking.

It appears as if the only possible explanation of these facts is the presence of a cytoplasmic system in the N-strain, this system being changed or *stimulated* by some factor transmitted *via* the sperm of the S-strain. This stimulation of the N-cytoplasm is not inherited to following generations but is repeated in every generation of line B and thereby prevents this line from coinciding with the S-strain.

The transmitted factor from the S-strain must also originate from some cytoplasmic system. Presumably this agent is diffusible and active in small quantities, and it is not necessary to assume that the cytoplasmic system itself is transmitted by the sperm. This agent has of course no effect on its own cytoplasm.

From the cytoplasm-substitutions it is evident that the corresponding system must be active in the N-strain as well, for the S-genotype in N-cytoplasm gives a higher reaction time than the pure S-strain. Comparison number 10 in Table 16 *b* further indicates that also the N-sperm transmits a stimulating effect to the S-strain, although of less effect. The interpretation thus postulates that as well normal strains (at least the more closely analysed N- and Or-strains) as the S-strain are provided with an active cytoplasmic system. In the S-strain, however, the system

is in some way changed or mutated. It decreases the sensitivity to anaesthetics in the same time as it produces some stimulating agent which, when transmitted to the N-strain induces activation of the corresponding normal system, resulting among other unknown things in an increased resistance to anaesthetics. How does this hypothesis agree with the other experiments? Series H from line B exceeds the pure S-strain in reaction time and is only a little below line B itself. Here the stimulating agent from the N-cytoplasm is transmitted by the sperm to the S-strain, and the N-cytoplasm continues to produce the stimulating agent also when the N-genome has been wholly substituted by the S-genotype. The stimulation does, however, not continue in the following outcrosses to S-animals series K and L, for there the N-cytoplasm is no longer involved and the stimulation is not inherited.

The phenomenon of series H from line A, which has a higher reaction time than line A itself during the first back-cross generations, must be supposed to have a similar cause. As long as part of the N-genotype persists in the A-line, the stimulating agent seems to be formed in spite of the absence of inherited N-cytoplasm. This stimulating agent is, as in the other cases, transmitted by the sperm to the S-egg where it produces the stimulation recorded in the higher reaction time of series H.

From line B it is evident that the cytoplasmic system from the N-strain is able to function and reproduce in the absence of the N-genes and must be regarded as autonomous.

The cytoplasm-substitution, comparison 7 and 8 in Table 16 *b*, show that such autonomy is not characteristic of the S-strain. Comparison 7 shows that an S-genotype in N-cytoplasm has no stimulating capacity and this is also clear from the crosses between N and B_{19}, which show no reciprocal differences. Comparison 8 in the same table shows that S-cytoplasm provided with an N-genotype also is without stimulating effect. In the S-strain both the original genotype and cytoplasm are necessary to produce the stimulating agent.

The stimulation of the autonomous cytoplasmic system in the N-strain causes the reaction time of line B to exceed that of the S-strain also when the whole N-genotype has been substituted. Why then does the line approach the S-level and not remain fixed at a higher level? It may be explained by the assumption that very occasionally cytoplasmic factors may be transmitted by the sperm and that the N-cytoplasm thereby becomes diluted by cytoplasmic factors from the S-strain. Such transmission is known to occur both in plants (transmission of plastids) and in animals (for instance, the CO_2-sensitive system in *Drosophila*).

When B_{19} is reciprocally crossed to the N-strain, none of the crosses exhibits a stimulation comparable with that in the N×S-cross. If the transmitted cytoplasmic S-system in B_{19} could function (which would be expected as the S-genotype is present) N×B_{19} ought to exceed B_{19}×N in reaction time, and the back-cross line B″ started from N×B_{19} ought to deviate from A″, started from B_{19}×N. This is not the case, and therefore it must be supposed that the stimulating agent from the cytoplasmic S-system either cannot be produced in the presence of N-cytoplasm or is produced in such small quantities that its effect cannot be detected. There is also no different trend for the back-cross lines A″ and B″. After 3 generations they have reached the level of line B, from which they originated, but for the number of generations that the lines are followed they do not coincide with the S-level. This is to be expected for line A″ where the rest of the original N-cytoplasm ought still to be stimulated, but for line B″ the cause is less evident. No observable stimulation takes place in the cross N×B_{19}, but in the first back-cross generation the sperm originates from the pure S-strain and ought to transmit a stimulating activity. That no difference from A″ is found in this or the following generations indicates that the cytoplasmic system in B″ cannot be stimulated to the full extent. The sperm from B_{19} must have changed the cytoplasm of the N-strain presumably depending on a mixed cytoplasmic origin.

An occasional transmittance of the cytoplasmic factors by the sperm can also be supposed to take place in line E, the back-cross line which restitutes the N-genotype after the cross S×N. E_{14} which was outcrossed reciprocally to the S-strain should then contain a mixture of S- and N-cytoplasm. This would be manifested in the outcrosses by a higher level in the cross E_{14}×S than in S×E_{14}. The reaction times found exceeded the S-level by 104 and 90 seconds, respectively. The difference is not significant and the back-cross lines for restitution of the S-genotype A′ and B′ reached the S-level simultaneously. If there has been a transmittance of N-cytoplasm, its stimulation must have been very weak.

As regards the cytoplasmic systems involved, two different phenomena must be distinguished. The first is the difference between the pure strains which depends on an interaction between genes and cytoplasm. This is evident from the tests of sensitivity made with the cytoplasm-substituted animals. The second phenomenon which complicates the analysis is the non-heritable stimulating effect which the two strains exert upon each other. Stimulation takes place when two conditions are fulfilled:

(1) A *stimulating agent* must be transmitted by the sperm.

(2) A *reactive system* must be present in the cytoplasm of the egg.

The stimulating agent from the S-strain changes the reactive cytoplasmic system of the N-strain. A co-operation between S-genotype and S-cytoplasm is necessary for the production of this agent. Sperms from males having an S-genotype in N-cytoplasm or an N-genotype in S-cytoplasm do not transmit any stimulating agent. The stimulating agent from the N-strain, the effect of which is seen in the two H-series, can on the other hand be produced as well by the N-cytoplasm associated with S-genotype as by the N-genotype in S-cytoplasm. Thus the cytoplasm is more gene independent in the N-strain.

The reactive system in the N-cytoplasm which is stimulated by the S-sperm is quite independent of the genotype present, and autonomous both as to function and reproduction. This is shown in line B, where the S-genotype is restituted, and in comparison 9 in the crosses with cytoplasm-substituted animals.

The N-genotype, however, cannot form its corresponding cytoplasmic system in the S-cytoplasm, shown by the fact that S-cytoplasm associated with N-genotype (line E) is not stimulated by S-sperms as the normal reactive system cannot be formed *de novo* by the N-genotype out of S-cytoplasm. Some change must thus have occurred in the S-cytoplasm, which cannot be compensated by the N-genotype. As the stimulating agent of the N-strain can be produced also in S-cytoplasm, it cannot be a product of the reactive cytoplasmic system, but rather a precursor of this.

About the reactive system in the S-strain the analysis does not afford any informations. Its effect is so slight that further informations concerning it would be difficult to obtain.

The nature of the nucleo-cytoplasmic systems which are involved in the anomaly of the S-strain is difficult to predict. It is evident from the determinations of ED50 and the experiments with repeated anaesthesia that the sensitivity can be influenced in different ways, and that the same phenotypic change can be produced by quite different processes. For instance, the reaction time of the N-strain may be reached both by introduction of the N-genotype in the S-cytoplasm and by repeated anaesthesia of S-animals with certain anaesthetics. But in the second case the value of ED50 is not changed and the increase of resistance disappears in a few hours.

As the anomaly of the S-strain is quantitative and can be recorded only by testing samples of many flies, comparisons with other analysed

cytoplasmic systems are complicated. Such systems are found which are of particulate nature, for instance the cytoplasmic systems in some yeast strains (EPHRUSSI, 1953), or they may be due to steady states of enzymes, as in the antigenic and mating types of *Paramecium* (discussion by DELBRÜCK in SONNEBORN and BEALE, 1949). They may even be some kind of a virus as the factor which causes the CO_2-sensitivity in *Drosophila* or the killer phenomenon in *Paramecium* (SONNEBORN, 1943). This is, however, unlikely in the present case, because of the negative results in the experiments with temperature treatment and transplantations.

If the experiments concerning respiration intensity had indicated differences between the pure strains, a quantitative change in some anaesthetic-sensitive oxidation system might have explained the different sensitivity to anaesthetics, in consistency with the findings of EPHRUSSI (1953), that many respiration enzymes in yeast are bound to self-reproducing cytoplasmic particles. This was, however, not the case, but significant differences were found between the pure strains and the F_1s, which might indicate that the stimulation process is in some way connected with oxidative processes. It might also be a result of the heterozygous condition in F_1. No enzymatic investigations have as yet been carried out on the material, and anyhow the consistent respiration of the pure strains show that the oxidative enzyme-systems cannot be the only ones involved.

ACKNOWLEDGEMENTS

I want to express my gratitude to my teacher, Professor GERT BONNIER, for his interest in my work and for many valuable discussions. I also want to thank Professor GÖTE TURESSON for his kind facilitation of my ordinary work during the time needed to finish the publication of this investigation.

Dr. K. G. LÜNING, Dr. A. HANSSON, Dr. B. HOLMSTEDT, Dr. A. NYGREN, and Mr. O. CLAESSON are heartily thanked for many fruitful discussions.

For technical assistance I am indebted to Miss. U. B. JONSSON and Mrs. B. CRONEBÄCK, and for revising the English text to Miss M. E. MAC KENDRICK.

The financial support from Naturvetenskapliga Forskningsrådet is gratefully acknowledged.

Finally, I want to thank my wife, Dr. M. RASMUSON, whose competent knowledge of genetics and statistics has been of great help to me.

SUMMARY

(1) A wild type bristle selected strain of *Drosophila melanogaster*, the S-strain, was found to be characterized by an outstanding sensitivity to ether and a rapid recover from anaesthesia. The inheritance of this anomaly was analysed by outcrosses to an inbred wild type strain, N, which reacted normally to anaesthetics.

(2) An experimental apparatus was constructed where groups of animals could be tested as to their sensitivity to anaesthetics, in most cases registered as the mean time to the moment of death. Two groups of flies could be tested simultaneously under identical conditions.

(3) The reaction times of the S- and N-strains were compared at different ages. The sensitivity of the strains deviated most at 24 hr. of age and least at 3 hr. of age. Young animals were less sensitive than the older ones. In comparisons to other wild type strains, the S-strain was always found to be the most sensitive. The sensitivity was not correlated to the mean weight per fly.

(4) The offspring from reciprocal crosses between the N- and S-strains deviated in sensitivity. The offspring from the cross N×S had a longer reaction time than any of the parental strains, the offspring from S×N coincided with the N-strain. Of other tested wild type strains some gave reciprocal differences, others did not.

(5) Lines of back-crosses were started from the reciprocal F_1s for restitution of the S- and N-genomes. Cross-bred females were mated to males from the parental strains. The sensitivity of the parental strains was rapidly restored in the back-cross line A, which restituted the S-genome from S×N and in both lines where the N-genotype was restituted, lines E and G. The back-cross line to S from N×S, line B, did not reach the sensitivity of the S-strain in 25 generations.

(6) If females from the S-strain were crossed to males from line B, the offspring had a higher reaction time than the S-strain. If S-females were crossed to males from earlier generations of line A, the offspring had a reaction time higher than line A itself.

(7) The 14th generation of line E was outcrossed reciprocally to the S-strain and new back-cross lines to S were started from the crosses. No reciprocal differences were found and the back-cross lines soon reached the sensitivity level of the S-strain. Correspondingly the 19th generation of line B was outcrossed to the N-strain and new back-cross lines started. No reciprocal differences were found. Both back-cross lines rapidly decreased to the level of line B from which they originated.

(8) Consistent results were attained in crosses and back-crosses when the wild type strain Oregon was used instead of N.

(9) Application of MATHER's method of chromosome assays revealed that gene differences between the S- and N-strains were present in all the major chromosomes. The gene dependent sensitivity can be regarded as polygenic.

(10) By outcrosses to a marked inversion stock it was possible to introduce the S-genotype into an N-cytoplasm. By counting the different segregating chromosome combinations it could be established that the S-genotype suffered no selective disadvantage in the N-cytoplasm. No difference in restitution rate can, therefore, be supposed for line A and B. The N-genotype could also be introduced into the S-cytoplasm by corresponding outcrosses. Comparisons between cytoplasm substituted and original strains showed that the N- and S-strains differed both in their cytoplasms and in their chromosomal genes. Crosses involving the cytoplasm substituted strains supported the results obtained in the back-cross lines.

(11) The mean effective dose (ED50) was determined for the pure strains and the reciprocal F_1s. Differences corresponding to those for reaction times were obtained. As ED50 can not depend on any difference in absorption rate, it is assumed that also the reaction times register the activity of some intracellular system.

(12) Different theories concerning the mode of action of anaesthetics have been reviewed. To test if the oxidative processes were correlated to the sensitivity, the respiration intensity was determined for N- and S-strains and the reciprocal F_1s. No differences were found between N and S or between the F_1s., but F_1 animals exceeded the parental strains significantly in respiration intensity.

(13) Tests with repeated anaesthesia revealed that diethyl and ethyl-vinyl ether induced a temporary increase of resistance, whereas divinyl ether and furan did not. The S- and N-strains reacted alike in this respect. The difference between the strains persisted after decapitation and in tests with other substances. It was not possible to change the sensitivity by means of temperature treatment or transplantation of haemolymph between the strains.

(14) It is concluded that the hereditary analysis of the differences in sensitivity is complicated by a mutual stimulation between the strains causing an increase of resistance. The stimulation takes place under two conditions, (1) a stimulating agent is transmitted by the sperm, and (2) a reactive system is present in the cytoplasm of the egg. The stimul-

ating agent in the S-sperm can only be produced by males provided with as well S-genotype as S-cytoplasm. The reactive system in the N-cytoplasm is autonomous both to reproduction and function, but cannot be synthesized by the N-genotype in S-cytoplasm. The opposite stimulation between N-sperm and S-cytoplasm is weaker and not so evident. The stimulating agent can be produced both by N-cytoplasm containing S-genotype and by N-genotype in S-cytoplasm.

Literature cited

BAUR, E. 1909. Das Wesen und die Erblichkeitsverhältnisse der »Varietates albomarginatae hort.» von *Pelargonium zonale*. — Z. ind. Abst. Vererb., 1: 330—351.

BUTLER, T. C. 1950. Theories of general anesthesia. — Pharmâcological Reviews, 2: 121—160.

CASPARI, E. 1936. Zur Analyse der Matroklinie der Vererbung in der a-Serie der Augenfarbenmutationen bei der Mehlmotte *Ephestia kühniella* Z. — Z. ind. Abst. Vererb., 71: 546—555.

— 1948. Cytoplasmic inheritance. — In Advances in Genetics, II: 1—66. New York: Academic Press, Inc.

CHEN, S. Y., EPHRUSSI, B. and HOTTINGUER, H. 1950. Nature génétique des mutants à déficience respiratoire de la souche B-II de la levure de boulangerie. — Heredity, 4: 337—351.

CORRENS, C. 1909. Vererbungsversuche mit blass(gelb)grünen und buntblättrigen Sippen bei *Mirabilis Jalapa, Urtica pilulifera* und *Lunaria annua*. — Z. ind. Abst. Vererb., 1: 291—329.

DELBRÜCK, M. 1949. Discussion in SONNEBORN, T. M. and BEALE, G. H.: Influence des gènes, des plasmagènes et du milieu dans le déterminisme des caractères antigéniques chez *Paramecium aurelia* (variété 4). — In Unités biologiques douées de continuité génétique. Édition du Centre Nat. Rech. Sci., Paris: 25—36.

DRILL, V. A. 1954. Pharmacology in Medicine. — New York: Mc Graw-Hill Book Comp., Inc.

EPHRUSSI, B. 1949. Action de l'acriflavine sur les levures. — In Unités biologiques douées de continuité génétique. Édition du Centre Nat. Rech. Sci., Paris: 165—180.

— 1953. Nucleo-cytoplasmic relations in micro-organisms. — Oxford: Clarendon Press.

EPHRUSSI, B. and HOTTINGUER, H. 1950. Direct demonstration of the mutagenic action of euflavine on baker's yeast. — Nature, 166: 956.

EPHRUSSI, B., HOTTINGUER, H. and CHIMENES, A. M. 1949. Action de l'acriflavine sur les levures. I. La mutation »petite colonie». — Ann. Inst. Pasteur, 76: 351—364.

EPHRUSSI, B., HOTTINGUER, H. and TAVLITZKI, J. 1949. Action de l'acriflavine sur les levures. II. Étude génétique du mutant »petite colonie». — Ann. Inst. Pasteur, 76: 419—450.

EDWARDS, G. A. 1953. Respiratory mechanisms. — In Insect Physiology, Chap. 4: 55—95. New York: John Wiley & Sons, Inc.

FISHER, R. A. 1925. Statistical methods for research workers. — Edinburgh: Oliver and Boyd.

GERARD, R. W. and HARTLINE, H. K. 1934. Respiration due to natural nerve impulses. A method for measuring respiration. — J. cellul. a. comp. Physiol., 4: 141—160.

GOLDSCHMIDT, R. 1924. Untersuchungen zur Genetik der geographischen Variation. I. — Arch. f. mikroskop. Anat. u. Entwicklungsmech., 101: 92—337.

— 1934. *Lymantria.* — Bibliogr. Genet., 11: 1—185.

GUILLEMAIN, A. and PLUS, N. 1953. Contrôle génique de la multiplication du virus de la sensibilité héréditaire au CO_2 chez *Drosophila melanogaster.* — Proceedings of the IX International Congress of Genetics. (In press.)

KIKKAWA, H. 1941. Mechanism of pigment formation in *Bombyx* and *Drosophila.* — Genetics, 26: 587—607.

— 1953. Biochemical genetics of *Bombyx mori.* — In Advances in Genetics, V: 107—140. New York: Academic Press, Inc.

KÜHN, A. 1927. Die Pigmentierung von *Habrobracon juglandis* ASHMED, ihre Praedetermination und ihre Vererbung durch Gene und Plasmon. — Nachr. Ges. Wiss. Göttingen: 407—421.

L'HÉRITIER, P. 1948. Sensitivity to CO_2 in *Drosophila.* A review. — Heredity, 2: 325—348.

L'HÉRITIER, P. and TEISSIER, G. 1937. Une anomalie physiologique héréditaire chez la *Drosophile.* — C. R. Acad. Sci., Paris, 205: 1099—1101.

L'HÉRITIER, P. and HUGON DE SCOEUX, F. 1947. Transmission par greffe et injection de la sensibilité héréditaire au gaz carbonique chez la *Drosophile.* — Bull. biol., 81: 70—91.

LILLIE, R. S. 1923. Protoplasmic action and nervous action. — Univ. of Chicago Press.

MAGNI, G. E. 1953. »Sex ratio»: a non-mendelian character in *Drosophila bifasciata.* Nature, 172: 81.

MATHER, K. 1942. The balance of polygenic combinations. — J. Genet., 43: 309—336.

MATHER, K. and HARRISON, B. J. 1949. The manifold effect of selection. — Heredity, 3: 1—52, 131—162.

MICHAELIS, P. 1942. Experimentelle Untersuchungen über die geographische Verbreitung von Plasmon-Unterschieden und der auf diese Unterschiede empfindlichen Gene, sowie deren theoretischen Bedeutung für das Kern-Plasma-Problem. — Biol. Zentr., 62: 170—186.

— 1947. Über das genetische System der Zelle. — Naturwissenschaften, 34: 18—22.

MICHAELIS, P. and BAKKER, D. 1948. Über reziprok verschiedene Sippenbastarde bei *Epilobium hirsutum.* VIII. Vergleichende Untersuchungen über das Plasmon mehrerer *Epilobium hirsutum*-Sippen, die bei reziproker Kreuzung Unterschiede der Pollenfertilität zeigen. — Z. ind. Abst. Vererb., 82: 384—414.

MILLER, A. 1950. The internal anatomy and histology of the imago of *Drosophila melanogaster.* — In Biology of *Drosophila,* Chap. VI: 420—534. New York: John Wiley & Sons, Inc.

MILLER, L. C. and TAINTER, M. L. 1944. Estimation of the ED50 and its error by means of logarithmic-probit graph paper. — Proc. Soc. Exp. Biol., 57: 261—264.

QUASTEL, J. H. and WHEATLEY, A. H. M. 1934. Narcotics and brain oxidations. Reversibility of narcotic action *in vitro.* — Biochem. J., 28: 1521—1529.

RHOADES, M. M. 1933. The cytoplasmic inheritance of male sterility in *Zea mays.* — J. Gen., 27: 71—93.

SNEDECOR, G. W. 1946. Statistical methods. — Ames, Iowa: The Iowa State College Press.

SONNEBORN, T. M. 1943. Gene and cytoplasm. I. The determination and inheritance of the killer character in variety 4 of *Paramecium aurelia*. II. The bearing of the determination and inheritance of characters in *Paramecium aurelia* on the problems of cytoplasmic inheritance, *Pneumococcus* transformation, mutations and development. — Proc. Nat. Ac. Sci., 29: 329—343.

— 1951. The role of the genes in cytoplasmic inheritance. — In Genetics in the 20th Century, Chap. 14: 291—314. New York: Mac Millan Comp.

STERN, C., SCHAEFFER, E. W. and SPENCER, W. P. 1944. The genetic basis of differences between two species of *Drosophila*. — Amer. Nat., 78: 183—188.

TRAUBE, J. 1904. Theorie der Osmose und Narkose. — Arch. f. d. ges. Physiol., 105: 541—558.

WINKLER, H. 1924. Über die Rolle von Kern und Protoplasma bei der Vererbung. — Z. ind. Abst. Vererb., 33: 238—253.

Contents

I. Introduction 147
II. Material and methods 149
1. Outline of culturing and collection of flies 149
2. Methods of testing the sensitivity to anaesthetics 151
A. Experimental apparatus 151
B. The dose 153
C. Sources of error 154
III. Experiments involving pure strains 155
IV. The sensitivity of the offspring from reciprocal crosses between the S-strain and different wild type strains 160
V. Back-crosses 165
1. Back-crosses to S-strain 165
2. Back-crosses to N-strain 170
VI. Outcrosses of the S-strain to the wild type strain Oregon 176
VII. Chromosome assays 177
VIII. Cytoplasmic interchange by outcrosses to a marked inversion stock 182
IX. Investigations concerning the mean effective dose (ED50) 185
X. Different theories concerning the mode of action of anaesthetics 189
XI. Experiments concerning the respiration intensity 191
XII. Additional experiments with the pure strains 193
1. Repeated anaesthesia 193
2. Miscellaneous experiments 197
XIII. Discussion 198
Acknowledgements 203
Summary 204
Literature cited 206

STRUCTURE, DIVISION AND DELIMITATION OF THE KINETOCHORE IN TRADESCANTIA

By *A. LIMA-DE-FARIA*

INSTITUTE OF GENETICS, UNIVERSITY OF LUND, SWEDEN

I. INTRODUCTION

A study of the chromosomes of *Agapanthus umbellatus* at the second division of meiosis revealed that the region of the chromosome body that holds together the two sister chromatids of each chromosome until the beginning of anaphase II is the most proximal region of the arms and not the kinetochore (LIMA-DE-FARIA, 1953 a and b). The treatment of root tips of *Allium Cepa* with colchicine showed also that at c-metaphase of mitosis the two sister chromatids of each chromosome are well separated from each other throughout most of their length, being only held together at the most proximal region of the arms and not at the kinetochore (LIMA-DE-FARIA, 1955 a). Subsequently the study of untreated mitosis in *Galtonia candicans* and *Vicia Faba* — where most chromosomes have one very short arm — permitted the finding that at the end of metaphase of mitosis the short and long arms of the two sister chromatids are firmly attached to each other while the two longitudinal halves of the kinetochore are distinctly separated and point to different spindle poles (LIMA-DE-FARIA, 1955 a).

After the study of this phenomenon at the second division of meiosis and at mitosis, an analysis of the first division of meiosis was required since SCHRADER (1939) studied the structure and division cycle of the kinetochore at this division and was led to conclude — like other authors — that the kinetochore was the region of the chromosome that was holding together the sister chromatids of each chromosome during the various stages of the first meiotic division.

II. MATERIAL AND METHODS

Anthers from flower buds of *Tradescantia virginiana* ($2n=24$) were fixed in acetic-alcohol 1 : 4 for two hours, transferred to 95 % alcohol where they were left over night, being subsequently stored in 70 % alcohol for a month. The preparations were made according to the

standard squash technique previously described (LIMA-DE-FARIA, 1948, 1952), the chromosomes being stained with iron-aceto-carmine. The preparations were made permanent using Canada balsam as mounting medium. No pre-treatment was used.

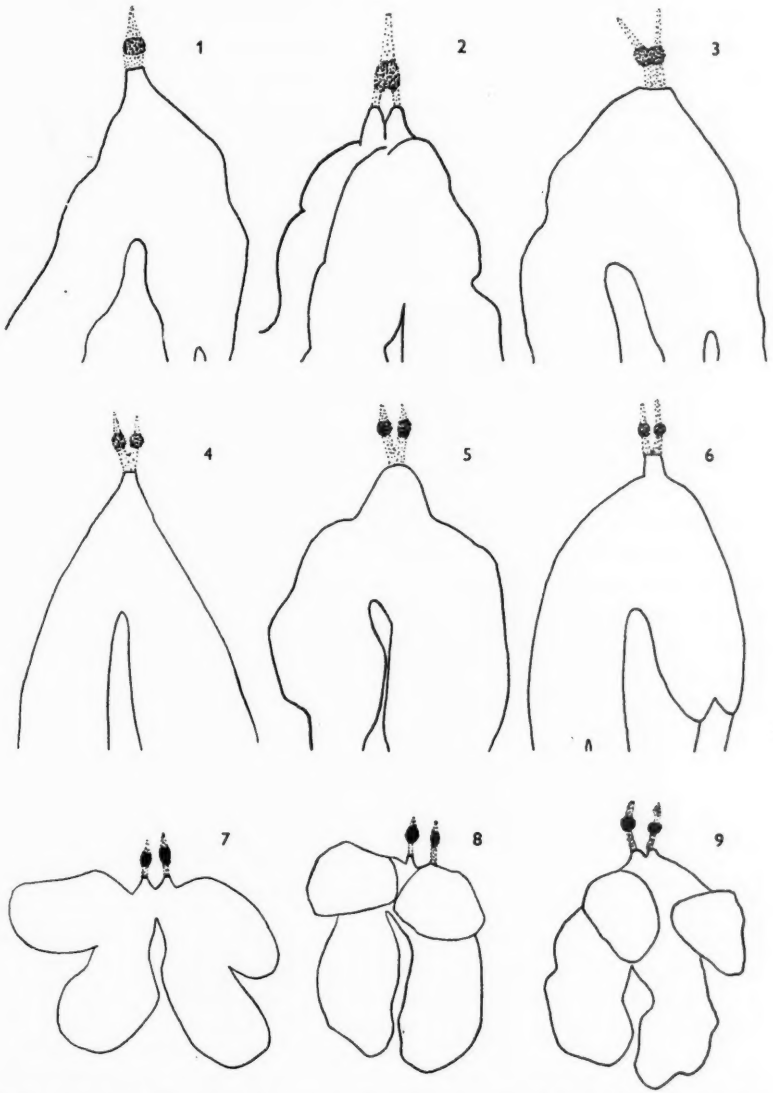
The plants studied contained, besides the chromosomes of the normal complement, several small chromosome derivatives. The results here described deal only with the structure and division cycle of the kinetochore in the chromosomes of the normal complement.

For taking the photomicrographs a Leitz »Universal-kameramikroskop Panphot» with a lens combination of 90× apochromatic oil immersion objective and an 8× periplanatic eyepiece was used. The negatives and positives were made employing Ilford Rapid Process Panchromatic plates and Ilford ultra hard glossy bromide paper respectively.

Besides *Tradescantia virginiana*, some plants of *Secale cereale* ($2n=14$) were used in this study to check certain structural details. The material of diploid rye contained B-chromosomes; one of the plants studied was from a Turkish strain while the other was from the same material as used in the pachytene analysis of the chromosome complement of rye (LIMA-DE-FARIA, 1952). The plants of tetraploid rye belonged to the variety Steel-rye cultivated by Professor A. MÜNTZING in 1949. The fixation and staining of the rye material were the same as used for *Tradescantia*.

III. STRUCTURE OF THE KINETOCHORE AT METAPHASE AND ANAPHASE I

At metaphase I of meiosis the kinetochore of the chromosomes of *Tradescantia virginiana* may be seen to be composed of three main zones (Fig. 1). A first exterior zone consisting of a slightly stained fibrilla, which establishes the connection between the arms and the other regions of the kinetochore. A second zone — the median zone — consisting of a distinct small body which has been called before the »spindle spherule» (SCHRADER, 1939) and which I prefer to call simply the spherule. My reservation towards such a designation rests on the fact that it is not known with certainty at present whether the contact of the kinetochore with the spindle includes its three regions or only this body. NAWASCHIN's and SCHRADER's drawings do not permit, in my opinion, to draw a definite conclusion on this question (NAWASCHIN, 1927; SCHRADER, 1939). The photomicrographs of IWATA



Figs. 1—9. ***Tradescantia virginiana.*** **— Figs. 1—3. Bivalents at middle metaphase I of meiosis (only one chromosome is represented). Fig. 1, the kinetochore undivided showing a weakly stained fibrilla (first zone), a spherule (second zone) and another weakly stained fibrilla (third zone). Fig. 2, apparent division of the first zone. Fig. 3, division of the third zone. — Figs. 4—6. Bivalents at late metaphase I exhibiting successive stages of the division of the kinetochore (only one chromosome is represented). — Figs. 7—9. Chromosomes at anaphase I showing the kinetochore completely divided. — All camera lucida drawings. — ×4500.**

(1940, Plate VII, Fig. 2) indicate that both the spherule and the interior zone of the kinetochore are in direct contact with the spindle fibres. The third zone is formed by a weakly stained fibrilla which has a terminal position but which constitutes the interior zone of the kinetochore (see below). The fixation and the staining used did not permit the spindle structure to be seen in these preparations. This last region is a part of the kinetochore and should not be confused with any spindle fibre which may or may not be in contact with it.

At middle metaphase I (Figs. 1—3) the spherules are usually not separated. The first zone is sometimes apparently divided and in a few cases it may be seen that the third zone is also divided (Figs. 2 and 3, respectively). For a discussion of the concept of division and a definition of this term, see LIMA-DE-FARIA, 1953 a. At late methaphase I (Figs. 4—6) the third region is distinctly divided, the two longitudinal halves being well separated from each other. At this stage the spherules are also clearly separated, but the first region is as a rule only divided in its upper part (Figs. 4—6). In Figs. 1—6 only one of the chromosomes that form a bivalent has been drawn.

At anaphase I the two longitudinal halves of the kinetochore, each belonging to one of the two sister chromatids that form a chromosome, may be seen well separated from each other throughout their whole length (Figs. 7—12). Fig. 10 represents a chromosome at anaphase I where its two constituent chromatids may be very well seen. Each kinetochore is apparently folded over itself since the kinetochore always makes the connection between both arms of a chromosome (or of a chromatid). One only distinguishes in Figs. 7—12 the transverse half of each kinetochore. One of the chromatids of a chromosome consists of: (1) the short arm attached to (2) one half of the kinetochore in upward direction, formed by one exterior zone, one spherule, one interior zone, (3) the other half of the kinetochore superimposed on the first one in downward direction, formed by one interior zone, one spherule and one exterior zone, and which is attached to (4) the long arm. The other chromatid has also a folded kinetochore composed by two halves one in upward and the other in a downward direction, each attached to one of the arms. The folding of the kinetochore cannot be seen in the pictures, which is an indication that the two halves lie closely superimposed (see below).

The structure of the kinetochore at anaphase I may be well seen in Figs. 7—9 and the respective photomicrographs (Figs. 10—12). In the three chromosomes the arms protrude slightly (see especially Figs. 7

Plate I. Fig. 10. *Tradescantia virginiana*, anaphase I of meiosis. Photomicrograph of the chromosome drawn in Fig. 7. The kinetochore is divided into two well separated longitudinal halves and one large spherule can be seen in each of them. Below and above each spherule are the weakly stained fibrillae of the exterior and interior zones, respectively. The demarcation line between the kinetochore and the arms may be well seen in one of the kinetochores. The short and long arms of each chromatid are in close contact at their proximal regions and the two sister chromatids are held together only at the proximal regions of both arms. — ×13500.

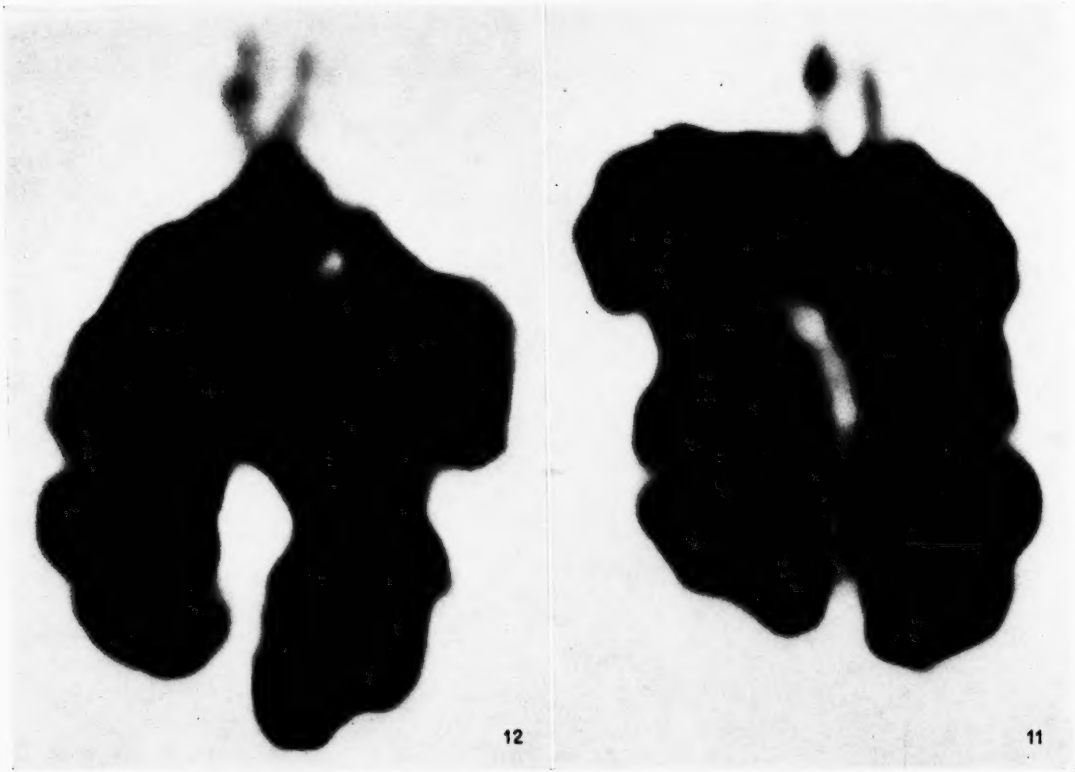


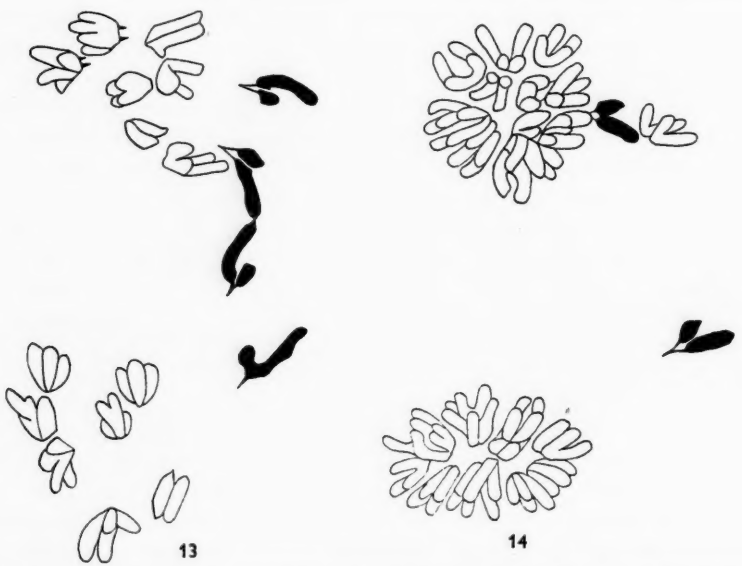
Plate II. Figs. 11—12. *Tradescantia virginiana*, anaphase I of meiosis. Photomicrographs of the chromosomes drawn in Figs. 8 and 9. respectively. — Fig. 11. In one of the kinetochores one large and dinstinct spherule is seen surmounting the weakly stained fibrilla of the exterior zone. The demarcation line between the kinetochore and the arms may be well seen in this chromatid. The other kinetochore is slightly out of focus. The two sister chromatids that constitute the anaphase chromosome are held together only at the most proximal regions of both short and both long arms (compare with Fig. 8). — Fig. 12. The three zones of the kinetochore may be seen quite distinctly in one of the kinetochores. The weakly stained fibrilla of the exterior zone is followed by a spherule. Over the spherule, the long and distinct weakly stained fibrilla of the interior zone is seen. The other kinetochore is also slightly out of focus. — ×11000.

and 10) and on the top of them the two kinetochores are seen. The three kinetochoric zones can be seen in each of the kinetochores of the chromosomes drawn in Figs. 7—9, but there is a variation in their size not only among chromosomes but also within the same chromosome. In Figs. 7 and 10 the two spherules are of unequal size and the interior zone (the terminal region) is rather small. A large and distinct spherule is seen in one of the kinetochores of Fig. 11. In this same picture the other spherule is not seen as large as in the camera lucida drawing (Fig. 8). This is due to difficulties in getting both spherules in focus at the same time. In Fig. 12 it can be seen distinctly in one of the kinetochores that the spherule has not a terminal position. The interior zone of the kinetochore (the terminal region in the picture) is quite long in this case. In the other kinetochore the spherule cannot be so well seen, also due to difficulties in getting both in focus (compare with Fig. 9).

Apparently the kinetochore diminishes in size from metaphase to anaphase. In Figs. 7—9 (anaphase I) it has nearly half the length and thickness that it has in Figs. 1—6 (metaphase I). At metaphase I the length of the folded kinetochore (that is, one half) is circa 2,0 micra and the diameter of the spherule circa 0,5 of the micron. At anaphase I the corresponding values are 1—1,5 micra and circa 0,3 of the micron, respectively. This means that at metaphase—anaphase I of meiosis the whole kinetochore of *Tradescantia* chromosomes has a length of 2—4 micra which is of the same order of magnitude as the length of the kinetochore of rye chromosomes at pachytene 2,5—3,6 micra (LIMA-DE-FARIA, 1952, Table 5).

The folding of the kinetochore at these stages was considered earlier by SCHRADER (1939, Fig. 1 c) but no attempt was made to demonstrate it. There are two main difficulties in the study of the folding of the kinetochore at metaphase and anaphase I of meiosis. One is due to the proximal regions of both arms of each chromatid being closely superimposed (Figs. 7—10), which is apparently related to the close superposition of the two kinetochoric halves. The other is due to the fact that once such a folding is observed in a chromosome it cannot be easily distinguished from a kinetochore in which the exterior zone is divided, as is for instance the case in Fig. 2.

A search was thus made for cases where free chromatids would be available at anaphase I for it was expected that in such cases these difficulties would be circumvented, but free chromatids are of rare occurrence at first anaphase of meiosis. In rye (*Secale cereale*) several cases of free sister chromatids were found and their study confirmed



Figs. 13—14. *Secale cereale*, anaphase I of meiosis in diploid (Fig. 13) and tetraploid rye (Fig. 14). — Fig. 13. Two of the non-sister chromatids of a bivalent (drawn in black) show difficulty of separation. The two other chromatids (drawn in black) have become precociously free and move to the spindle poles. In the two separated sister chromatids of the upper anaphase group the kinetochore can be seen to be folded and uniting the short and long arm. The other 6 chromosomes of the normal complement and one B chromosome are seen in each anaphase group. — Fig. 14. Division of one univalent into its two sister chromatids (drawn in black). In the chromatid of the lower anaphase group the kinetochore can distinctly be seen to be folded and uniting the short and long arm. The two arms are not in a so intimate contact at their proximal regions as in the case of undivided chromosomes. Camera lucida drawings. — ×2500.

the observations made in *Tradescantia*. Bivalents may sometimes have difficulties to separate in the regions where they normally form chiasmata, and as a consequence the non-sister chromatids may remain attached until mid-anaphase or later (Fig. 13). If one pair of non-sister chromatids is strongly attached and the other not, the active mobility of the kinetochore is apparently sufficient to release one of the sister chromatids from its other sister chromatid winning the resistance made by the connection at the most proximal region of the arms (see below). This phenomenon was observed in two different plants of rye and was found in one and four cells, respectively. In these cells the two

sister chromatids of each chromosome of one of the bivalents became separated and free chromatids could be studied at anaphase I (Fig. 13). It can be clearly seen in the two separated sister chromatids of the upper anaphase group that the kinetochore is folded and distinctly connects the short with the long arm. As well, the superposition of the arms was here not so intimate as in the bivalents, which helped to put in evidence this phenomenon.

In tetraploid rye (Fig. 14) univalents are formed at the first division of meiosis where they may be found to divide (MÜNTZING, 1951). This results also in the formation of free sister chromatids (or daughter-univalents). In these the kinetochore can be clearly seen to be folded, making the connection between the short and long arm (Fig. 14).

A second division of the kinetochore, corresponding to a division into half-sister chromatids — as indicated by the figures of NEBEL (1935) and SCHRADER (1939) — is not to be confused with the folding of the kinetochore, since: (1) the chromatids are seen in »side view» and not with the short arm lying behind the long arm, (2) they are not divided or individualized into half-sister chromatids, and (3) the connection is distinctly between the most proximal region of the short arm and the most proximal region of the long arm (Figs. 13 and 14). Most significant is that a similar folding could be seen in *Tradescantia gigantea* at the beginning of anaphase of the first pollen mitosis as seen clearly in Fig. 2 b of PROPACH's work (PROPACH, 1940).

IV. THE DIVISION CYCLE OF THE KINETOCHORE

At anaphase I of meiosis the division of the kinetochore is very distinct (Figs. 10—12) and the region that holds together the two sister chromatids is the proximal region of the arms as may be seen especially well in Figs. 7, 8, 10, and 11. The connection between the two sister chromatids is at the proximal regions of both the short and long arms as may be seen in Figs. 8 and 11.

SCHRADER (1939) saw in *Tradescantia reflexa* the spherules distinctly divided but he was led to assume that the kinetochore was still undivided at anaphase I. As it was supposed at that time that the kinetochore was the region that held together the two sister chromatids of each chromosome until the end of metaphase II of meiosis, he was led to assume that what he called the »cup or commissural mass» — and which he believed to be a region of the kinetochore — was responsible for holding the two sister chromatids together. The existence of the

»cup» was deduced from his studies in *Amphiuma* — where the chromosomes were stained with mitochondrial stains or with haematoxylin which was later strongly extracted — and SCHRADER himself was not quite convinced that it represented the binding element of the chromatids (SCHRADER, 1939, p. 236). It is now clear from the study here made that the two longitudinal halves of the kinetochore (corresponding each to one of the two chromatids) are separated far apart at anaphase I of meiosis, and that the binding element of the two sister chromatids is the proximal region of the arms.

The kinetochore undergoes during meiosis a large variation in its appearance. Whereas here at metaphase I it is a structure that protrudes from the arms, at prophase II it is (at least in *Agapanthus* and other materials) a weakly stained region that lies flat between both arms. It is, thus, not surprising that at prophase II of meiosis the two longitudinal halves of the kinetochore may be seen individualized but lying side by side, as described in *Agapanthus* (LIMA-DE-FARIA, 1953 a), although they may be separated from each other at the end of the previous division.

As described above (Fig. 13), in cases where at anaphase I the chromosomes of a bivalent show difficulty in separation, one of the sister chromatids of a chromosome may detach from the other sister chromatid, moving singly to the spindle pole. This and other similar phenomena have been described by many authors as involving a precocious division of the kinetochore (DOWRICK, 1953; ÖSTERGREN, 1951; DARLINGTON, 1939, Fig. 11; GEITLER, 1938; RICHARDSON, 1936; and others). Led by this belief, DOWRICK (1953) designated as »precentric» the precocious separation of the sister chromatids in the chromosomes of *Chrysanthemum* at anaphase I of meiosis. It is, however, clear that in *Tradescantia virginiana* — and probably in many other organisms — the kinetochore is already divided at anaphase I. Also, in *Secale cereale* the kinetochore is already divided at anaphase I as may be seen in two of the chromosomes of the upper anaphase group of Fig. 13 and the same can be seen in *Trillium Smallii* in the figures of IWATA (1940, Fig. 7 and Plate VII, Fig. 6). What takes place at this stage under these particular conditions is a precocious division of the most proximal region of the arms and not of the kinetochore.

V. THE DELIMITATION OF THE KINETOCHORE

In the study of the kinetochore one of the main problems has been to establish with accuracy the structures which represent the kine-

tochore at the various stages of mitosis and meiosis and also to establish the homologies between the various kinetochoric zones at these stages. There were two main difficulties: (1) the accurate delimitation of the kinetochore and (2) the investigation of its structural components. Several authors have confused the proximal regions of the arms — which are often positively heteropycnotic — with the kinetochore, and others have confused the kinetochore itself, as seen at anaphase I of meiosis, with the spindle fibres. On the other hand, as a well differentiated structure of the kinetochore has been lacking for a long time the establishment of the homologies was most difficult.

It is now, however, possible to establish these homologies with accuracy. The concept of kinetochore springs originally from the »primary constriction» seen in mitotic chromosomes at metaphase. If we do not limit the kinetochore to this region, it is not possible to establish any other well defined limits for it, and the distinction between kinetochore and arms breaks down.

The finding of a well differentiated structure within the kinetochore of the pachytene chromosomes of rye and *Agapanthus* (LIMA-DE-FARIA, 1949, 1950) was significant not only because it described a complex structure at the kinetochore but also because the limits of the kinetochore could be defined with accuracy in these chromosomes. On both sides of the kinetochore the arms exhibit a conspicuous gradient in chromomere size. The position and morphological features of the region thus delimited at pachytene as the kinetochore correspond with the position and morphological features of the »primary constriction» seen at metaphase of mitosis. The finding of TJIO and LEVAN (1950) was also significant not only because it described in mitotic chromosomes a well differentiated structure within the kinetochore, corresponding to that seen at pachytene in rye and *Agapanthus* but also because the »primary constriction» where this structure was found could be delimited with accuracy. The treatment employed by these authors did not affect the structure of the chromosomes in any drastic way. This was not so in the case of the observations made by LEVAN, STEINEGGER, ÖSTERGREN, MAKINO and MOMMA where the treatments employed were so violent that an accurate delimitation of the kinetochore could not be made in most cases (LEVAN, 1946; LEVAN and STEINEGGER, 1947; STEINEGGER and LEVAN, 1947; ÖSTERGREN, 1947; MAKINO and MOMMA, 1950).

The deeply stained structures that LEVAN (1946) described at mitosis in several *Allium* species, and which suffered a strong chemical treatment, correspond to the most proximal region of the arms and not to

the kinetochore. The deeply stained bodies that he saw in *Dipcadi serotinum* at prophase of mitosis were similar to those that he observed in this species in the most proximal regions of the arms at telophase and in the regions that were in contact with the nucleolus at prophase of mitosis. ÖSTERGREN (1947) first homologized his findings with LEVAN's and identified with the most proximal region of the arms the structures that he saw in contact with the spindle fibres. After my observations in rye (LIMA-DE-FARIA, 1949) he homologized the same structures with the kinetochoric chromomeres (ÖSTERGREN, 1951). The treatment employed by him had so drastic effects — the chromosome body being »often completely dissolved» — that such a change of homology clearly exemplifies the difficulties in limiting the kinetochore under such conditions. Among other effects, the treatment with chloroform of the root-tip chromosomes of *Allium Cepa* and *Colchicum autumnale*, made by STEINEGGER and LEVAN, resulted in the near dissolution of the chromosome body, the kinetochore being apparently the last region to disappear. The chromosomes were so completely dissolved and the structures observed had such a minute size that it must have been difficult to decide whether they represented the most proximal regions of the arms or the kinetochore (STEINEGGER and LEVAN, 1947, Figs. 1 *r* and 1 *s*, and LEVAN and STEINEGGER, 1947, Fig. 1 *i*). It is, however, immaterial, in connection with the study of the structural differentiation of the kinetochore, to know whether they represent one or the other of these regions, for no particular differentiation was found within what was called the kinetochore. In the case of *Colchicum* mitotic chromosomes treated with acenaphthene, the deeply stained regions described as the kinetochore may as well be the proximal regions of the arms (LEVAN and STEINEGGER, 1947, Fig. 1 *k*). The observations made in grasshopper chromosomes at both mitosis and meiosis by MAKINO and MOMMA (1950), after pre-treatments with cold and hot water, led them to describe as the kinetochore the deeply stained regions which they saw at the ends of the arms. These regions did not show any structure that could be considered to correspond to that of the kinetochore.

Similarly SCHRADER's study in the meiotic chromosomes of *Amphiuma* — where the chromosomes were stained with mitochondrial stains or with haematoxylin which was later strongly extracted — suffers from the same difficulty (SCHRADER, 1936, 1939). SCHRADER had no safe criterion which permitted him to delimit the kinetochore exactly, and what he described as »spindle spherules» is most probably the most proximal region of the arms. Bodies similar in size and aspect to those

that SCHRADER described as spindle spherules could be seen by him in the interphase nuclei and looking as pro-chromosomes usually do (SCHRADER, 1936, Plate I, Fig. 3 and 1939, Fig. 4 b). Pro-chromosomes are known to correspond in most organisms to the positively heteropycnotic and proximal regions of the arms and not to the kinetochore, or to other positively heteropycnotic regions of the arms (*cf.* HEITZ, 1932; DARLINGTON, 1937; STEBBINS, 1950; TJIO and LEVAN, 1950; LIMA-DE-FARIA, 1954 a).

The evidence obtained from the studies of LIMA-DE-FARIA (1949, 1950) and of TJIO and LEVAN (1950) permitted to homologize with accuracy the region delimited at metaphase of mitosis as the kinetochore with the region delimited at pachytene with the same name. On the other hand the delimitation of the kinetochore at first metaphase and first anaphase of meiosis continued to be an uncertain question. NEBEL (1935) correctly delimited the kinetochore at this last stage but SCHRADER (1939), IWATA (1940), MATSUURA (1941), and ÖSTERGREN (1951) contributed to increase the uncertainty on this point by including a part of the arms in what they called the kinetochore. NEBEL and SCHRADER worked with *Tradescantia reflexa* and ÖSTERGREN with *Tradescantia virginiana* (the species here studied) and *T. paludosa.* SCHRADER (1936, 1939) included in the kinetochore what he called the »cup or commissural mass» owing to the fact that at this time it was supposed that it was the kinetochore that was holding together the sister chromatids of a chromosome until the beginning of the second anaphase of meiosis (SCHRADER, 1939, p. 236). As he saw the spherules divided he thought that some other element of the »kinetochore» should be holding them together and included in it this region of the arms. IWATA (1940) was inclined to homologize with the spherules the pro-chromosomes that he saw at interkinesis and telophase II of meiosis in *Trillium Smallii.* MATSUURA (1941) included in the kinetochore of the chromosomes of *Trillium kamtschaticum* the deeply stained region that he saw at metaphase I of meiosis in the most proximal region of the arms after pre-treatment with water. He considered this region as being composed of a kind of matrix involving the negatively heteropycnotic kinetochore. MATSUURA described in his *Trillium* material the fibrillae of the exterior zone and the spherules of the median zone of the kinetochore but he considered the latter as an »artefact». Having as his main object the study of chromosome movements, ÖSTERGREN (1951) did not carry out a sufficiently detailed analysis of the structure of the kinetochore at metaphase I of meiosis to permit him to delimit with accuracy

the kinetochore at this stage. Sometimes the spherules and the other fibrillar regions of the kinetochore were considered as *a part* of the »kinetochore» (pp. 109 and 110), in other cases they were not included in the »kinetochore», or considered as something accessory, since the »kinetochore» was called isopycnotic (p. 93) and in still other cases they were called »kinetochore» (p. 101, Figs. 15 and 17). Moreover he did not define the limits of what he called the »kinetochore region» (*e. g.*, p. 102) nor did he refer to any criterion by which such a region could be delimited. ÖSTERGREN (1951) was thus led to include in the kinetochore the proximal region of the arms, and for this reason to describe as isopycnotic the »kinetochore region» or »kinetochore» of *Tradescantia virginiana* (ÖSTERGREN, 1951, pp. 102 and 93). As can be seen from Figs. 10—12, the kinetochore is in *T. virginiana* negatively heteropycnotic at anaphase I and it has the same degree of heteropycnosis at metaphase I (Figs. 1—6).

Whereas SCHRADER (1939), IWATA (1940), MATSUURA (1941), and ÖSTERGREN (1951) had no evidence sufficient to enable them to set precise limits to the kinetochore at the first metaphase and anaphase of meiosis, the structural analysis here carried out permits accurate delimitation of the kinetochore at these stages. This delimitation is based on five distinct sources of evidence.

(1) There is a sharp demarcation line between the deeply stained arms and the weakly stained structure protruding from them (Figs. 7—12). Especially in Fig. 10 it can be clearly seen that this structure lies between the short and the long arm of each chromatid as the kinetochore always does. This structure is also negatively heteropycnotic (Figs. 7—12), as the kinetochore usually is at pachytene and metaphase of mitosis. The distinctness of the boundary between the arms and the kinetochore is accentuated by the fact that the exterior zone of the kinetochore has a fibrillar structure (Figs. 10, 11 and 12).

(2) The three zones of the kinetochore found both at pachytene in rye and *Agapanthus* (LIMA-DE-FARIA, 1949, 1950) and at metaphase of mitosis in many species belonging to such different families as the Commelinaceae, Liliaceae, Araceae, Amaryllidaceae, Gramineae, and Papilionaceae (TJIO and LEVAN, 1950) are also present in the structure here delimited as the kinetochore. These zones have approximately the same size and keep the same relative positions that they have at the other stages. The kinetochore of *Tradescantia virginiana* has at metaphase—anaphase I of meiosis approximately the same length that it has at pachytene in rye.

(3) This homology cannot only be established between the structure of the kinetochore of *Tradescantia virginiana* and the structure of the kinetochore of other unrelated species but can also be made by comparing the structure of the kinetochore within the genus *Tradescantia.* The same essential type of kinetochoric structure described here for *T. virginiana* at metaphase—anaphase I was found by TJIO and LEVAN (1950, Fig. 1 a) at metaphase of mitosis in root tips of *T. bracteata* and by PROPACH in the prophase and metaphase chromosomes of the first pollen mitosis of *T. gigantea* (PROPACH, 1940, Figs. 1, 2 a and 2 b). In *T. bracteata* (a species closely related to *T. virginiana*)[1] an exterior zone composed of a weakly stained fibrilla, a median zone where a spherule can be seen and an interior zone consisting also of a weakly stained fibrilla, constitute the transverse half of the kinetochore of each sister chromatid at metaphase of mitosis, just as they do here at anaphase I in *T. virginiana.* In these three cases the kinetochore is negatively heteropycnotic and its limits can be established with accuracy. SCHRADER (1939) studied the structure of the kinetochore in *T. reflexa* at metaphase and anaphase I of meiosis but described the spherule as having a terminal position, although he has drawn the spindle fibres not in contact with it (SCHRADER, 1939, Fig. 5 a). In *T. virginiana* the spherule does not have a terminal position and a distinct third zone situated above the spherule (the interior zone) can be observed in most cells (Figs. 1—10 and especially Fig. 12). This same interior zone could be distinctly seen by TJIO and LEVAN (1950, Fig. 1 a) at metaphase of mitosis in *T. bracteata.*

(4) In rye it could be demonstrated that the region delimited at metaphase I as the kinetochore corresponds to the region delimited at pachytene with the same name. In this species a chromosome derivative was found with a deleted kinetochore (LIMA-DE-FARIA, 1954 b, 1955 b). At pachytene the deleted kinetochore had about one third the length of the kinetochore of the normal chromosomes and the corresponding part of its structural elements. At metaphase I this same chromosome derivative exhibited a kinetochore that was also about one third shorter and thinner than the kinetochore of the normal chromosomes. The kinetochoric structure observed at metaphase I in rye is essentially similar to that observed here in *Tradescantia* at the same stage.

(5) Finally, the evidence on the division cycle of the chromosome body should be used as an additional element in the delimitation of the

[1] I am indebted to Mr. B. PETERSON for systematic information concerning these species.

kinetochore. It can be distinctly seen, especially in Fig. 10, that immediately after the limit of the kinetochore the arms of the two chromatids are firmly held together. It has been demonstrated that in a wide number of organisms this region that holds the chromatids together is the most proximal region of the arms, and not the kinetochore (LIMA-DE-FARIA, 1953 a, 1955 a). Evidence of the same phenomenon in *Tradescantia* is to be found in the work of PROPACH where he has drawn at early anaphase of the first pollen mitosis the two longitudinal halves of the kinetochore well separated and pointing to different spindle poles, whereas the arms were still kept together throughout most of their length (PROPACH, 1940, Figs. 2 b and 4). At pollen mitosis the connection between the arms starts just after the limit of the kinetochore as here at anaphase I.

VI. SIMILARITIES AND DIFFERENCES BETWEEN THE KINETOCHORE AT METAPHASE AND ANAPHASE OF MITOSIS AND AT METAPHASE AND ANAPHASE I OF MEIOSIS

The comparison that follows is one simply based on what so far one has been able to observe in the kinetochore of *Tradescantia.*

Similarities:

(1) As described above, in the root tips of *Tradescantia bracteata* (TJIO and LEVAN, 1950, Fig. 1 a) each transverse half of the kinetochore of a sister chromatid exhibits at metaphase of mitosis (a) an exterior zone consisting of a weakly stained fibrilla, which makes the connection with the arms, (b) a median zone consisting of a spherule and (c) an interior zone composed of a weakly stained fibrilla. In *T. virginiana* at metaphase and anaphase I of meiosis the same three zones can be seen (Figs. 1—12). Their relative size and position are the same as at metaphase of mitosis.

(2) At both the metaphase of mitosis studied in root tips and at first metaphase and anaphase of meiosis the kinetochore is negatively heteropycnotic. This is due to the fibrillar nature of its components and the small size of the bodies there found. In *Tradescantia gigantea* (PROPACH, 1940) the kinetochore is also negatively heteropycnotic at metaphase of the first pollen mitosis.

Differences:

(1) In *T. gigantea* (PROPACH, 1940, Fig. 2 b) at the beginning of anaphase of the first pollen mitosis the kinetochore of each sister chromatid can be seen to be folded, but the two halves of each kinetochore diverge considerably at the base — the proximal regions of the short and the long arms remaining a certain distance apart. In *T. virginiana* at anaphase I of meiosis the kinetochore of each sister chromatid is apparently folded. But here the contact between the two halves is so intimate that the folding cannot be seen (Figs. 1—12). On the other hand it can be seen that the short and long arms of each chromatid are very closely superimposed at their proximal regions, leaving no space between them at this point (Figs. 10—12).

(2) The position of the kinetochores relative to the spindle poles is also different in the two types of division. At early anaphase of mitosis the two kinetochores — each belonging to one of the sister chromatids — are both located in a plane that is parallel to the spindle axis. At anaphase I of meiosis the two kinetochores are located in two different planes that are parallel to the spindle axis. In the first case the two kinetochores point to different spindle poles, in the second case they point to the same spindle pole. To what this difference is due cannot be decided at present. ÖSTERGREN (1951, p. 110) already pointed out this difference but what is here delimited as the kinetochore he considered to be only its »kinetic components».

SUMMARY

(1) At metaphase and anaphase I of meiosis the kinetochore of the chromosomes of *Tradescantia virginiana* may be seen to be composed of three zones: an exterior zone consisting of a weakly stained fibrilla, a median zone consisting of a spherule and an interior zone where a weakly stained fibrilla can be seen. This is the same type of structural pattern as previously found in other organisms at pachytene and metaphase of mitosis.

(2) At metaphase and anaphase I of meiosis the kinetochore is apparently a folded structure where both halves are closely superimposed since the kinetochore always makes the connection between both arms of a chromosome (or of a chromatid) and since the protruding structure is identical with a transverse half of the kinetochore as seen at pachytene and metaphase of mitosis. The close contact of the two kineto-

choric halves and of part of the arms is an obstacle to the analysis of the folding.

(3) Confirmation that the kinetochore is folded at anaphase I of meiosis was obtained in free sister chromatids studied at this stage in rye. In these cases the difficulties present in the study of complete chromosomes are circumvented and the kinetochore can be seen to be folded, uniting both arms of a chromatid.

(4) In *Tradescantia virginiana*, at anaphase I of meiosis, the region that holds together the sister chromatids of each chromosome is the most proximal region of both arms. The kinetochore is already divided at this stage.

(5) The delimitation of the kinetochore in the chromosomes of *T. virginiana* at metaphase and anaphase I can now be made with accuracy for: (a) there is a sharp demarcation line between the weakly stained kinetochore and the deeply stained arms, (b) the three kinetochoric zones found at pachytene and at metaphase of mitosis in many different organisms are not only present at these stages but they have also the same relative size and position, and (c) immediately after the limit of the kinetochore appears the region which holds the sister chromatids together and which is known to be, in *Tradescantia* and other organisms, a part of the arms. Moreover, in rye it could be demonstrated that the region delimited at metaphase I as the kinetochore corresponds to the region delimited at pachytene with the same name.

(6) At both metaphase of mitosis and metaphase and anaphase I of meiosis the kinetochore is negatively heteropycnotic in *Tradescantia* chromosomes. The degree of the kinetochoric folding and the position of the kinetochores relative to the spindle poles are different at early anaphase of mitosis and at anaphase I of meiosis.

Literature cited

DARLINGTON, C. D. 1937. Recent advances in cytology. — London. 671 p.

— 1939. Misdivision and the genetics of the centromere. — J. Genetics 37: 341—364.

DOWRICK, G. J. 1953. The chromosomes of *Chrysanthemum*, III: Meiosis in *C. atratum*. — Heredity 7: 219—226.

GEITLER, L. 1938. Weitere cytogenetische Untersuchungen an natürlichen Populationen von *Paris quadrifolia*. — Zschr. indukt. Abst.- u. Vererb. 75: 161—190.

HEITZ, E. 1932. Die Herkunft der Chromocentren. Dritter Beitrag zur Kenntnis der Beziehung zwischen Kernstruktur und qualitativer Verschiedenheit der Chromosomen in ihrer Längsrichtung. — Planta 18: 571—636.

IWATA, J. 1940. Studies on chromosome structure. II. The attachment chromomeres in the meiotic chromosomes. — Jap. J. Bot. X: 375—382.

LEVAN, A. 1946. Heterochromaty in chromosomes during their contraction phase. — Hereditas XXXII: 449—468.

LEVAN, A. and STEINEGGER, E. 1947. The resistance of *Colchicum* and *Bulbocodium* to the c-mitotic action of colchicine. — Hereditas XXXIII: 552—566.

LIMA-DE-FARIA, A. 1948. B chromosomes of rye at pachytene. — Portug. Acta Biol. (A) 2: 167—174.

— 1949. The structure of the centromere of the chromosomes of rye. — Hereditas XXXV: 77—85.

— 1950. The Feulgen test applied to centromeric chromomeres. — Hereditas XXXVI: 60—74.

— 1952. Chromomere analysis of the chromosome complement of rye. — Chromosoma 5: 1—68.

— 1953 a. The regions of special cycle of division of *Agapanthus* chromosomes. — Chromosoma 6: 33—44.

— 1953 b. Structure and properties of the kinetochore. — Proc. IXth Int. Congr. Genetics. (In the press.)

— 1954 a. Chromosome gradient and chromosome field in *Agapanthus*. — Chromosoma 6: 330—370.

— 1954 b. Pachytene analysis of a chromosome derivative of centromere size. — Proc. VIIIth Int. Congr. Botany. Rapports et Communications, Section 9, p. 42.

— 1955 a. The division cycle of the kinetochore. — Hereditas 41: 238—240.

— 1955 b. Structure and behaviour of a chromosome derivative with a deleted kinetochore. — Chromosoma 7: 51—77.

MAKINO, S. and MOMMA, E. 1950. Observations on the structure of grasshopper chromosomes subjected to a new acetocarmine treatment. — J. Morph. 86: 229—252.

MATSUURA, H. 1941. Chromosome studies on *Trillium kamtschaticum* Pall. XIII. The structure and behavior of the kinetochore. — Cytologia 11: 369—379.

MÜNTZING, A. 1951. Cyto-genetic properties and practical value of tetraploid rye. — Hereditas XXXVII: 17—84.

NAWASCHIN, S. 1927. Zellkerndimorphismus bei *Galtonia candicans* Des. und einigen verwandten Monokotylen. — B. Deut. Bot. Gesellschaft 45: 415—428.

NEBEL, B. R. 1935. Chromosomenstruktur VI. Ein Ausschnitt. — Züchter 7: 132—136 and 155—156.

PROPACH, H. 1940. Die Centromeren in der Pollenkornmitose von *Tradescantia gigantea* ROSE. — Chromosoma 1: 521—525.

RICHARDSON, M. M. 1936. Structural hybridity in *Lilium Martagon album*×*L. Hansonii*. — J. Genetics 32: 411—450.

SCHRADER, F. 1936. The kinetochore or spindle fibre locus in *Amphiuma tridactylum*. — Biol. Bul. 70: 484—498.

— 1939. The structure of the kinetochore at meiosis. — Chromosoma 1: 230—237.

STEBBINS, G. L., JR. 1950. Variation and evolution in plants. — New York, 643 p.

STEINEGGER, E. and LEVAN, A. 1947. The cytological effect of chloroform and colchicine on *Allium*. — Hereditas XXXIII: 515—525.

TJIO, J. H. and LEVAN, A. 1950. The use of oxiquinoline in chromosome analysis. — Anal. Est. Exp. Aula Dei 2: 21—62.

ÖSTERGREN, G. 1947. Proximal heterochromatin, structure of the centromere and the mechanism of its misdivision. — Bot. Not. 1947: 176—177.
— 1951. The mechanism of co-orientation in bivalents and multivalents. The theory of orientation by pulling. — Hereditas XXXVII: 85—156.

Contents

I. Introduction 209
II. Material and methods 209
III. Structure of the kinetochore at metaphase and anaphase I 210
IV. The division cycle of the kinetochore 215
V. The delimitation of the kinetochore 216
VI. Similarities and differences between the kinetochore at metaphase and anaphase of mitosis and at metaphase and anaphase I of meiosis 222
Summary 223
Literature cited 224

INVESTIGATIONS ON HAPLOID RAPE

By *GÖSTA OLSSON* and *ARNE HAGBERG*
SVALÖF, SWEDEN

INTRODUCTION

IN a field of flowering rape (*Brassica napus* L.) a few abnormal plants are regularly found. Their anthers do not open, and the plants have a decreased fertility or are nearly completely sterile. Some of these plants also have smaller flowers than the normal plants. As a whole, their morphology indicates that they may be haploids. Such spontaneous, haploid mutants of rape have been briefly described by MORINAGA and FUKUSHIMA as early as 1933.

For practical breeding work it is of interest to ascertain the causes of sterility in the rape populations. Thus, the presumably haploid plants ought also to be thoroughly investigated. According to U (1935) and others, rape contains the genomes of turnip rape and cabbage, and can be synthesized by crossing these in combination with a doubling of the chromosome number. One of the projects in Swedish oil plant breeding is to synthesize a winter-hardy rape by crossing winter-hardy types of turnip rape and cabbage. Therefore, it is important to compare the F_1 of this cross with haploid rape, especially with regard to the behaviour at meiosis.

MORPHOLOGY

Using the size of flowers as guidance, 7 plants have been selected as presumably haploids during the period 1950—1953. Of these plants, 4 have been found in new élites of Svalöf's Matador winter rape, 2 have been found in propagations and in plots in field trials at Svalöf, and 1 was found in the F_1 material from a cross between two rape varieties. All seven plants have grown under such conditions that it is impossible for them to have originated from spontaneous crossings betwen cabbage and turnip rape.

The common rape, grown as an oil crop, has the chromosome number $2n=38$. The seven plants with small flowers have 19 chromosomes each, as has been checked by squash preparations of small leaves and of somatic bud cells.

It is difficult to obtain a correct estimate of the frequency of haploid seeds formed in a normal rape population. Probably, some haploids will be removed by the seed cleansing. And later on, the smaller and weaker haploid plants will be suppressed and perhaps killed in the competition with the normal and strongly growing diploid plants in the field populations. At flowering time we tried, however, to estimate the frequency of haploids in a field of winter rape. In a 6000 square metre propagation field of the rape variety Svalöf's Matador, 3 haploids were found by careful and thorough investigation.

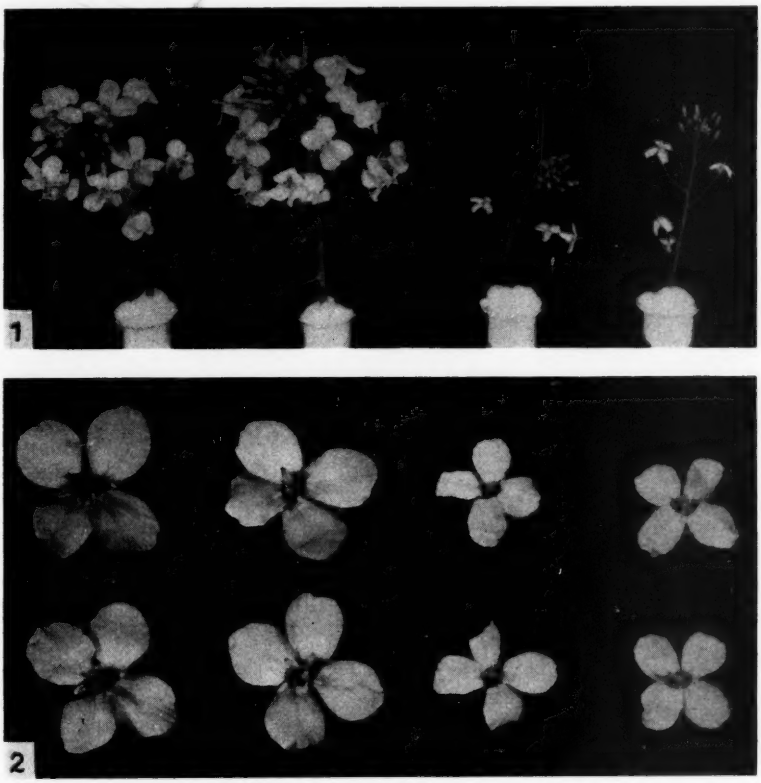
The morphology of the haploids very much resembles that of the diploids. The haploid rape plants are, however, of a more slender type than the diploids. The length of stomata in the haploids is significantly shorter than in the corresponding diploids, as will be seen in Table 1.

TABLE 1. *Length of stomata in haploid and diploid rape.*

Degree of ploidy	Length of stomata in scale units (means from 20 cells per plant)									Number of plants	Mean value	
	5	5,5	6	6,5	7	7,5	8	8,5	9		scale units	my
Haploid	1		2	3		1				7	6,11	21,1
Diploid						1	2	3	1	7	8,04	27,7

Other types of cells have not been studied. The cells are, however, probably smaller throughout the haploids than in the diploids. Different organs are smaller in size and look more slender in the haploids than in the diploids. This is especially noticeable in the case of stems, buds and flowers (Figs. 1 and 2). In the diploids the length and breadth of the petals are 9,0 and 7,7 mm, respectively, while the corresponding values in the haploids are 6,7 and 4,9 mm.

The haploids are almost totally sterile. The anthers do not open. But if they are squashed, a few well-developed pollen grains may be found (about 0,1—0,5 per cent). Parts of the plants have been isolated in bags; no seeds have been obtained. On non-isolated parts of the plants the flowers have been supplied with excess of pollen from diploid rape plants; such flowers may grow to small pods with one or two seeds. In this case we obtain an estimate of the frequency of functioning female gametes. About half a per cent of the ovules are developed into seeds. Some of these seeds have been sown, and from them fully fertile, diploid rape plants with 38 chromosomes have grown up. A few plants with about 57 chromosomes have, however, also been found among the progeny plants from the haploids. KOMATSU (1936)



Figs. 1 and 2. Flowers of diploid (to the left) and haploid rape.

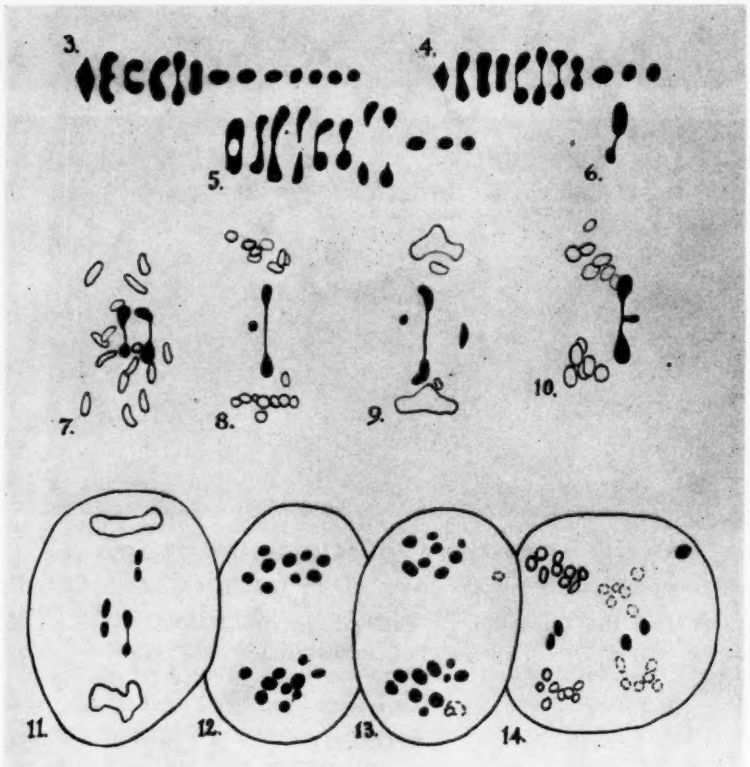
has studied the formation of embryo-sacs in haploid rape and the progeny from this haploid, but unfortunately his paper was not available.

CYTOLOGY

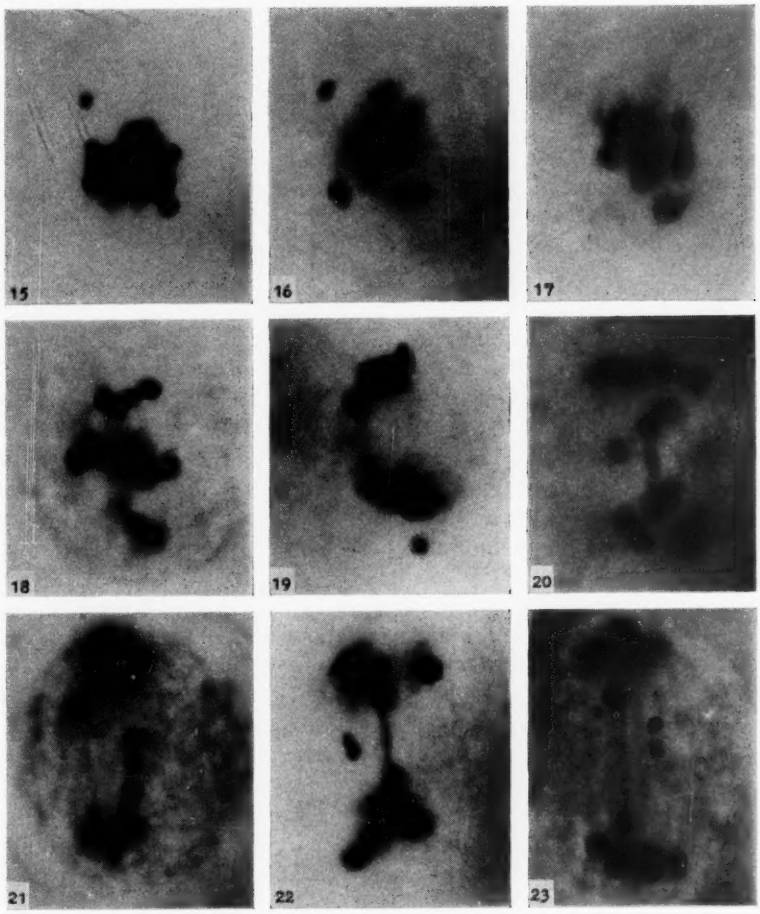
Meiosis has been studied in four of the haploid plants. Buds were fixed in Carnoy and in Karpechenko after prefixation in Carnoy. Slides were made which have been stained in the classical way with crystal violet. The different stages of meiosis from first metaphase to the formation of tetrads have been studied.

TABLE 2. *Meiosis of haploid rape, configurations at metaphase I.*

Plant No.	Number of bivalents									Sum of PMC investigated
	0	1	2	3	4	5	6	7	8	9	
1950—1					1	3	6	5	5		20
1952—403—1			1	1	2	9	14	12	9	2	50
1952—1					1	1	2	3	3		10
1953—316—1				2	3	3	6	3	3		20
Sum of PMC			1	3	7	16	28	23	20	2	100



Figs. 3—14. Different stages of meiosis in haploid rape. — 3—5. Chromosome configurations in three typical PMC at M_I. — 6. Heteromorphic bivalent. — 7. PMC at A_I with 2 bivalents and 15 univalents. — 8. PMC at A_I with bridge and fragment. — 9. Ditto+lagging univalent. — 10. PMC at A_I with a bridge due to delayed terminalization. — 11. Dividing univalents at T_I. — 12. M_{II}-plates with 9 and 10 chromosomes, respectively. — 13. M_{II}, see text. — 14. A_{II} with laggards.



Figs. 15—23. Different stages of meiosis in haploid rape. — 15—17 M_I; 17 shows a heteromorphic bivalent. — 18—23. A_I showing laggards and bridges. — 18. PMC with 4 divided bivalents and 11 univalents lagging. — 21. One of the bivalents shows delayed terminalization.

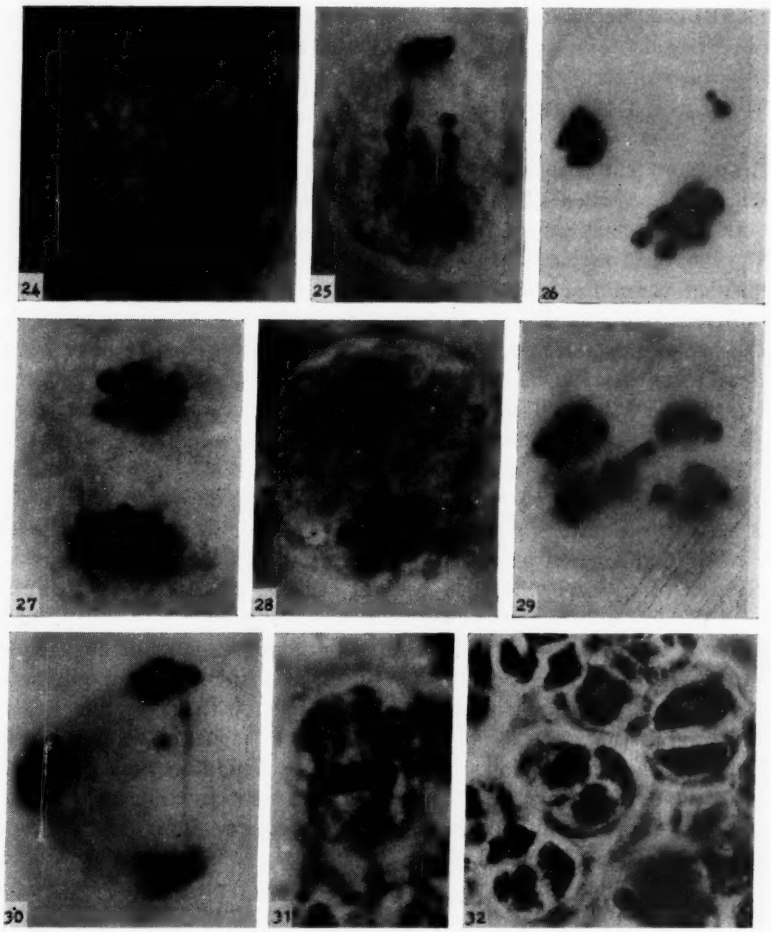
Meiosis in haploid rape has previously been briefly described by MORINAGA and FUKUSHIMA (1933), thus: »In the metaphase of the heterotypic division, a variable number, ranging from 0—6 or 7, of bivalents of stretched forms are found. When no bivalent is formed, which is rather rare in this haploid, the distinction of meta- and early

anaphase becomes obscure». In our studies we have found a higher frequency of bivalents. Thus, most of the pollen mother cells (PMC) have 5—8 bivalents (Table 2 and Figs. 3—5 and 15—17). In two PMC there are probably 9 bivalents (possibly $8_{II}+3_{I}$) and in one cell only as low a number of bivalents as two have been found (Fig. 7). During a special search for cells without bivalents a few such cells were found at the bottom of the thecas (in the proximal parts). The frequency of cells with 19 univalents is, however, very low and it is sometimes difficult to distinguish cells with 19 univalents from cells in an anaphase stage. One or two ring bivalents are normally found (Fig. 5), the others are rod bivalents. Heteromorphic bivalents are also to be found (Figs. 6 and 17). Thus, chromosomes of unequal size are paired. Trivalents or tetravalents have not been observed with certainty.

At first anaphase some PMC have bridges with fragments (Figs. 8—9 and 23—24). Some of these »first division bridges» persist in the second division, at second metaphase (Fig. 28) as well as at second anaphase (Fig. 29). In the second division bridges also occur, as can be seen in Fig. 30. All these bridges indicate structural differences between the paired chromosomes, most of them probably inversions. Very often a special type of bridge is found at first anaphase. They are without fragments and probably caused by delayed terminalization, due to non-homologous segments at the subterminal or terminal parts of the paired chromosomes (*cf.* MAC KEY, 1954; HAGBERG, 1954; ÅKERMAN and HAGBERG, 1954). (Figs. 10 and 19—22.) Thin threads between chromatids in dividing univalents (Figs. 11 and 23) were observed in most PMC at first anaphase and such formations are to be expected, as explained by ÖSTERGREN (1951, p. 116).

Some of the univalents are included in the anaphase groups without division, others will be auto-oriented and divide at late anaphase and telophase. A few univalents lie peripherically in the cell plasm (Fig. 26) and are not included in the spindles of the second division.

The chromosomes which can be studied in the second metaphase plates are not equivalent. As an example, in Figs. 13 and 27 seven chromosomes in each plate may come from seven bivalents. In the upper plate there is also one chromatid from a divided univalent. In the lower plate, however, three chromatids are found and one undivided univalent. Two univalents lie in the cell plasm entirely outside the plates. Checking the number of chromosomes — bodies — in second metaphase plates is, therefore, of less value; they are given for comparison with other papers (*e. g.*, MORINAGA and FUKUSHIMA, 1933)



Figs. 24—32. Different stages of meiosis in haploid rape. — 24—25. A_I and T_I with laggards and a bridge. — 26. M_{II} with two chromosomes definitely outside the plates. — 27. M_{II}, see text. — 28. M_{II} with a bridge persisting from A_I. — 29. A_{II} with a bridge persisting from A_I. — 30. T_{II} with bridge and laggard. — 31. M_{II}, side view, 19 chromosomes dividing, giving rise to a dyad. — 32. A dyad (to the right) among tetrads.

in Table 3. By chance, the mean frequency 9,5 bodies at second metaphase is exactly the theoretically expected value. But there are two hidden errors which influence the mean value in opposite directions.

TABLE 3. *Meiosis of haploid rape, configurations at metaphase II.*

	Number of bodies in one of the metaphase plates									Number of PMC	Mean number of bodies
	6	7	8	9	10	11	12	13	14		
Frequency	1	3	15	36	30	8	5	1	1	100	9,5

The elimination of univalents decreases the number of bodies; and the division of univalents into chromatids increases the number of bodies.

At second anaphase, irregularities are also to be found as bridges (Fig. 30) and laggards (Fig. 14). Most of the laggards are probably chromatids from univalents divided at first anaphase. As they have no ability to divide once more during meiosis, some of them may possibly undergo misdivision; configurations indicating misdivision have been seen. Thus, the tetrads may contain a very varying number of chromosomes and they will not grow to viable pollen grains. In some tetrads micronuclei are found but they are not very common.

In a few cells — at the proximal end of the theca — at second metaphase there was found only one plate containing 19 chromosomes, all of which have probably been univalents. These PMC may have formed a restitution nucleus at the first division; and in this case the second division of meiosis will be a normal somatic division, giving pollen with the haploid number of chromosomes. (Fig. 31.) U (1935, p. 408) writes: »These are obviously due either to the regression which occurred in the heterotypic division or to the complete union of two spindles». Normally, tetrads are formed but these PMC with restitution nuclei form dyads (Fig. 32). These dyads occur in a frequency corresponding to that of the well developed pollen grains. And the functioning gametes of haploid rape have, as a rule, the same chromosome number as those of normal, diploid rape since the progeny from haploid rape consists mainly of normal, diploid plants.

DISCUSSION

As a rule, a very low frequency of bivalents is found at meiosis of haploids of common diploid species which with certainty are not polyploids (*cf.* LEVAN, 1942 and 1945). KATAYAMA (1935) made the distinction: »the haploid from diploid species (or basal species) is called a *monohaploid*. If the haploid had occurred from allopolyploids, it is classified under the name of *polyhaploid*». For monohaploids LEVAN (1945, p. 409) maintains: »that the chromosome pairing in haploids depends on purely chromosome-mechanical factors

and that as a rule the chiasmata formed should not be taken as proof of homologies within the genome». The actual plants of haploid rape are, however, polyhaploids derived from an amphidiploid species and they contain one *oleracea-* and one *campestris*-genome. Possibly the term *amphihaploids* is a more adequate one for this special type of polyhaploids. The relatively high frequency of bivalents occurring in haploid rape cannot be caused by »purely chromosome-mechanical factors» only. As is sometimes the case in polyhaploids, there is certainly a partial homology between the paired chromosomes. No pairing occurs between chromosomes of the *campestris* genome, as shown by RAMANUJAM (1941). His monohaploid plant of *B. campestris* ssp. *toria* showed solely univalents at first metaphase. Only a few PMC with some bivalents were found. Monohaploid *B. oleracea* is not studied, as far as we know. However, the F_1 hybrid between white mustard and cabbage has no bivalents at meiosis (U, NAGAMATU and MIDUSIMA, 1937) and, thus, there is no reason to expect pairing between the different cabbage chromosomes. The bivalents at meiosis of amphihaploid rape are composed of one *campestris-* and one *oleracea*-chromosome each. This chromosome pairing, moreover, is due to the occurrence of a partial homology two-by-two between practically all the chromosomes in the genomes of cabbage and turnip rape. In some other polyhaploids there is practically no pairing between chromosomes from different genomes as, for instance, in haploid wheat (GAINES and AASE, 1926). For further discussion of conjugation of chromosomes in different polyhaploids, we may refer to the review of the problem of haploidy by KOSTOFF (1942).

The amphihaploids may be of value in studying what happens to amphidiploids during evolutionary establishment. RAMANUJAM and SRINIVASACHAR (1943) write: »In the haploid *B. juncea*, the pairing of chromosomes between the subgenomes is very much less than that in the F_1 hybrid *B. campestris*×*B. nigra* with which the haploid should correspond. The reduction in the pairing of chromosomes in the haploid may have been brought about by the elimination of cross-homology between the sub-genomes of *B. juncea* since the establishment of the amphidiploid condition». Now, it is possible to compare the meiosis in amphihaploid rape by that in the F_1 hybrid between *B. campestris* and *B. oleracea*. This hybrid was investigated by U (1935) for the first time. He found 0—8 bivalents at first metaphase and 8—12 — as a mean 9,6 — chromosomic bodies at second metaphase. Thus, there is a very good agreement in meiotic behaviour of this hybrid and the

amphihaploid rape plants studied here. Thus, in *B. napus* we have not found the same difference between amphihaploid and F_1 hybrid as in the case of *B. juncea* cited above. Possibly rape is a younger amphidiploid than *B. juncea*, but, as far as we know, there is no reason for such an assumption.

The haploid rape plants described here seem to form less unreduced gametes than the haploids studied by MORINAGA and FUKUSHIMA where »fairly often the heterotypic division is suppressed», or than U's hybrid where »one large nuclear plate with 19 or 20 chromosomes was frequently met with at homotypic metaphase». This last-mentioned hybrid also had a better pollen fertility (7,4 per cent of good pollen) than the polyhaploids (0,1—0,5 per cent). The hybrid which was kept living during two flowering seasons gave some self-fertilized seeds without bag isolation during the second year. In the first year it was quite sterile.

It may also be possible to obtain seeds after self-fertilization of haploid rape. There may be methods for increasing the frequency of unreduced gametes. Nor can we exclude the possibility of propagating the haploids vegetatively and of treating buds with colchicine to get totally homozygous, diploid rape plants. For practical reasons, however, it is impossible to check the haploids as seedlings and to treat the plumula with colchicine. Of course, it would be of importance for the practical rape breeding to find methods to obtain homozygous, pure lines of rape. This may, as discussed above, be possible if we could get self-fertilized seeds of haploid rape plants, which are not too rare in the populations, or if we could double their chromosome number.

SUMMARY

Seven haploid plants of rape (*Brassica napus* L.) have been found and are described as to morphology and cytology. The frequency of haploid plants occurring in rape populations may be illustrated by the fact that in a field with rape, 6000 m^2 in size, 3 haploid plants were found. The haploids are slender plants, which are nearly fully sterile. From (0), 2—8 or 9 bivalents are found at metaphase I of meiosis. Heteromorphic bivalents occur. Different types of meiotic irregularities are discussed. A few dyads are formed in about the same frequency as good pollen grains. The progeny of haploids contain normal diploids and a few plants with about 57 chromosomes. The possibility to obtain homozygous diploid rape plants is discussed.

The investigation has been supported by grants from the Sales Association of the Manufactures of Oleomargarine.

Literature cited

GAINES, E. F., and AASE, H. C. 1926. A haploid wheat plant. — Am. Jour. Bot., 13: 373—385.

HAGBERG, A. 1954. Cytogenetic analysis of erectoides mutations in barley. — Acta Agr. Scand., IV: 472—490.

KATAYAMA, Y. 1935. Karyological comparisons of haploid plants from octoploid *Aegilotricum* and diploid wheat. — Jap. Jour. Bot., VII: 349—380.

KOMATSU, T. 1936. Investigations in progeny of haploid plants and embryosac formation by diploid and haploid plants of *Brassica Napus*. (Japanese.) — Proc. Crop Sci. Soc. Japan, 8: 364—372.

KOSTOFF, D. 1942. The problem of haploidy. — Bibliogr. Genet., XIII: 1—148.

LEVAN, A. 1942. Studies on the meiotic mechanism of haploid rye. — Hereditas XXVIII: 177—211.

— 1945. A haploid sugar beet after colchicine treatment. — Hereditas XXXI: 399—410.

MAC KEY, J. 1954. Neutron and X-ray experiments in wheat and a revision of the speltoid problem. — Hereditas XL: 65—180.

MORINAGA, T. and FUKUSHIMA, E. 1933. Karyological studies on a spontaneous haploid mutant of *Brassica Napella*. — Cytologia, 4: 457—460.

RAMANUJAM, S. 1941. A haploid plant in *Toria* (*Brassica campestris* L.). — Proc. Indian Acad. Sci., XIV: 25—34.

RAMANUJAM, S. and SRINIVASACHAR, D. 1943. Cytogenetic investigations in the genus *Brassica* and the artificial synthesis of *B. juncea*. — Indian Jour. Genet. Pl. Breed., 3: 73—88.

U, N. 1935. Genome-analysis in *Brassica* with special reference to the experimental formation of *B. napus* and peculiar mode of fertilization. — Jap. Jour. Bot. 7: 389—452.

U, N., NAGAMATU, T. and MIDUSIMA, U. 1937. A report on meiosis in the two hybrids, *Brassica alba* RABH. ♀×B. *oleracea* L. ♂ and *Eruca sativa* LAM. ♀×*B. oleracea* L. ♂. — Cytologia, FUJII Jub. Vol.: 437—441.

ÅKERMAN, Å. and HAGBERG, A. 1954. Intraspecific sterility in oats. — Hereditas XL: 438—452.

ÖSTERGREN, G. 1951. The mechanism of co-orientation in bivalents and multivalents. — Hereditas XXXVII: 85—156.

Communications from the Swedish Seed Association, No. 87.

THE DIVISION CYCLE OF THE KINETOCHORE

By *A. LIMA-DE-FARIA*

INSTITUTE OF GENETICS, UNIVERSITY OF LUND, SWEDEN

THE view held at present by most cytologists is that the region of the chromosome body that holds together the two sister chromatids of a chromosome until the end of metaphase of mitosis and until the end of metaphase II of meiosis is the localized kinetochore or centromere (DARLINGTON, 1937, 1946; SHARP, 1943; SCHRADER, 1953; WHITE, 1954).

(1) At prophase and metaphase II of meiosis the two sister chromatids of each chromosome are in most materials widely separated from each other throughout most of their length, a phenomenon that permits accurate checking of the region at which they are held together. A study of the chromosomes of *Agapanthus umbellatus* at the second division of meiosis revealed that as early as pro-metaphase II the two longitudinal halves of the kinetochore are distinctly separated from each other (Figs. 1—4). The region of the chromosome body that holds together the two sister chromatids of each chromosome until the beginning of anaphase II is the most proximal region of the arms and not the kinetochore (LIMA-DE-FARIA, 1953).

(2) It is not to be expected that this phenomenon could be easily studied at prophase and metaphase of mitosis, owing to the close association of the two chromatids of each chromosome throughout most of their length. However, if after the use of a particular treatment the chromatids were found to be widely separated from each other for most of their length, this phenomenon might be analysed also at mitosis. This is the case in materials treated with colchicine and other chemicals where X-shaped chromosomes appear. The treatment of root tips of *Allium Cepa* with 0,01 % colchicine solution during 32 hours revealed clearly the same phenomenon. The cells were stained with aceto-orcein and mounted in Canada balsam after dehydration in the usual way (LIMA-DE-FARIA, 1952). At c-mitotic metaphase the two sister chromatids of each chromosome are well separated from each other throughout most of their length, being only held together at the most proximal region of the arms and not at the kinetochore (Figs. 5—8). At this stage,

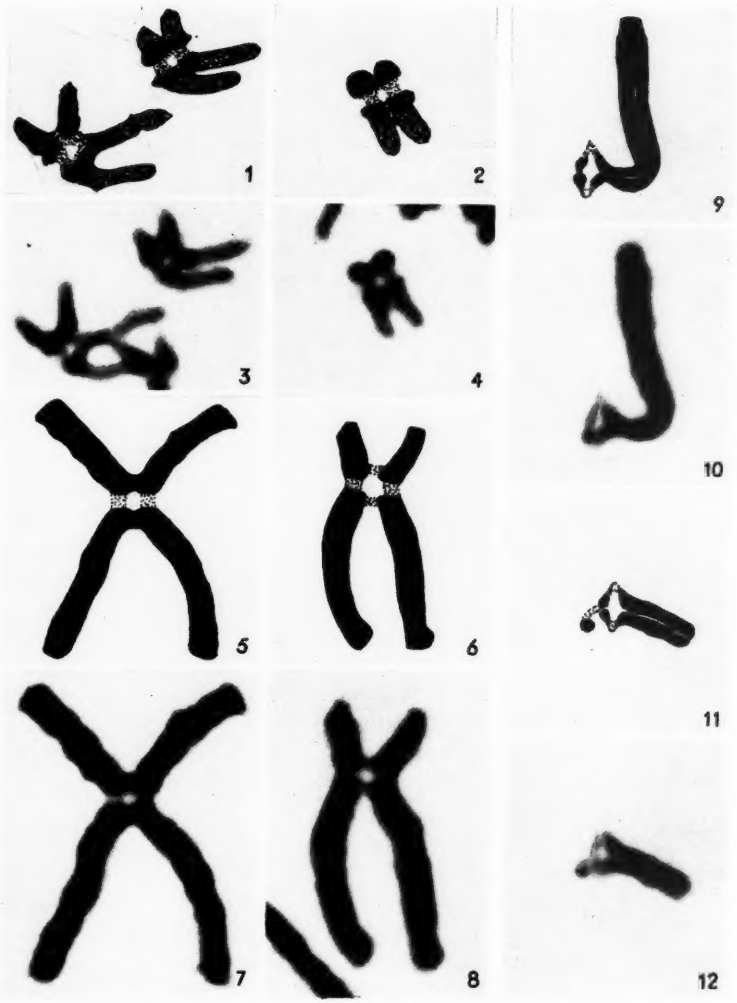


Plate I. Figs. 1—12. — Figs. 1—4. Chromosomes of *Agapanthus umbellatus* at prometaphase II of meiosis (from LIMA-DE-FARIA, 1953). ×2500. — Figs. 5—8. Chromosomes of *Allium Cepa* at metaphase of mitosis after colchicine treatment. Figs. 5—6 ×4500, Figs. 7—8 ×5000. — Figs. 9—12. Chromosomes of *Galtonia candicans* at metaphase of mitosis (untreated). — Figs. 11 and 12, the satellite chromosome. ×2500. — Figs. 1, 2, 5, 6, 9, and 11, camera lucida drawings, other Figs. are photomicrographs of the same chromosomes. The two longitudinal halves of the kinetochore are separated from each other in all cases, while the two sister chromatids are still held together by the arms.

the two longitudinal halves of the kinetochore are distinctly separated from each other (Figs. 5—8). C-anaphase starts when the two sister chromatids fall apart at the most proximal region of the arms.

(3) Subsequently the possibility was contemplated of studying the same phenomenon under normal conditions. In chromosomes with subterminal kinetochore the very short arms may have a length which equals that of the most proximal region of the long arms. This is the case in *Galtonia candicans* where every chromosome of the complement has a very short arm. A study of the root tip mitosis of this species revealed that at the end of metaphase the long arms of the two sister chromatids are individualized throughout most of their length but are kept together by a weakly stained structure or substance, and the very short arms can also be seen to be quite firmly attached to each other by a similar structure or substance, while the two longitudinal halves of the kinetochore are distinctly separated and point to different spindle poles (Figs. 9—12). When anaphase starts the short arms separate subsequently and the long arms separate last. In *Vicia Faba* where most chromosomes of the complement have one very short arm it can also be seen at metaphase of mitosis that the two longitudinal halves of the kinetochore are already separated from each other whereas the very short and long arms of the two sister chromatids still keep in close contact at this stage.

Although it seems that this phenomenon has never been described or given detailed treatment in the literature, a search through the drawings and photomicrographs of a number of authors shows it clearly both at the second division of meiosis, and at the normal and treated mitosis in a wide number of organisms. The reason may be found in the difficulties of studying the phenomenon during the normal mitosis, in a misconstruction of the concept of division (as confusion between individualization and separation), and in pre-conceived ideas on the properties and structure of the kinetochore.

My attention was recently called to a paper of GEITLER (1951), where he refers to the division of the kinetochore previous to the division of the arms at mitosis in a species of Diatoms, but this author still believes that after colchicine treatment it is the kinetochore that holds the sister chromatids together, and no reference is made to the second division of meiosis. Most important is that GEITLER does not know which region of the chromosome body is responsible for holding together the sister chromatids.

Although the kinetochore is not, as a rule, the region responsible for

keeping together the sister chromatids of a chromosome until the end of metaphase of mitosis and until the end of the second metaphase of meiosis, it has, however, a cycle of division of its own, distinct from that of the other regions of the chromosome body. Some of the implications of this phenomenon have been discussed previously (LIMA-DE-FARIA, 1953).

Literature cited

DARLINGTON, C. D. 1937. Recent advances in cytology. — London. 671 pp.
— 1946. The evolution of genetic systems. — Cambridge. 151 pp.
GEITLER, L. 1951. Der Bau des Zellkerns von *Navicula radiosa* und verwandten Arten und die präanaphasische Trennung von Tochtercentromeren. — Österr. Botan. Zeitschr. 98: 206—214.
LIMA-DE-FARIA, A. 1952. Chromomere analysis of the chromosome complement of rye. — Chromosoma 5: 1—68.
— 1953. The regions of special cycle of division of *Agapanthus* chromosomes. — Chromosoma 6: 33—44.
SCHRADER, F. 1953. Mitosis. — New York. 170 pp.
SHARP, L. W. 1943. Fundamentals of cytology. — New York. 270 pp.
WHITE, M. J. D. 1954. Animal cytology and evolution. — Cambridge. 454 pp.

SYNTHESIS OF THE ssp. RAPIFERA OF BRASSICA NAPUS

By *G. OLSSON, A. JOSEFSSON, A. HAGBERG* and *S. ELLERSTRÖM*

THE SWEDISH SEED ASSOCIATION, SVALÖF, SWEDEN

SEVERAL investigations have been carried out regarding the relationship between species within the genus *Brassica*. Of particular importance is the work on amphidiploidy in *Brassica* carried out by the Japanese MORINAGA, U and others during the first half of the thirties. Their papers elucidate in a remarkable way the relationship between some *Brassica* species. Thus, U (1935) has shown that *B. napus* was obtained from the cross between *B. oleracea* and *B. campestris*. This was later verified by several geneticists.

B. oleracea comprises several distinctly different subspecies and most of them have been used to produce artificial *B. napus*. Also within *B. campestris* s. lat. different subspecies are to be differentiated (OLSSON, 1954). In crosses with *B. oleracea* all these subspecies, according to U (1935, p. 422), ought to raise amphidiploid *Brassica napus*.

By using different types of *B. oleracea* and *B. campestris* as parents in the crosses there ought to be possibilities to obtain different types of *B. napus*. Hitherto ssp. *oleifera* has most often been used as the *campestris* parent in the crosses, and *oleifera* forms of *napus* have then been obtained. MIZUSHIMA (pers. comm.) in his crosses, however, has also used a leafy *campestris* type (ssp. *pekinensis*) from which he obtained an artificial *napus* suitable for feeding.

CRANE (1943) believes that swedes (*B. napus* ssp. *rapifera*) are formed by a cross between *oleracea* (perhaps kohlrabi or marrow stem kale) and turnips. But he does not present any data to support this. To find the degree of crossability between the species such cross experiments were carried out by CALDER (1937) — marrow stem kale×turnips — and by BECKER (1951, 1952) — kohlrabi×turnips. None of them got any F_1 plants from these crosses. KOCH and PETERS (1952/53) have crossed turnips with kale, thereby getting a winter-hardy rape (HOFFMANN, pers. comm); but they give no data as to the type of root. The present investigation is a new attempt to produce swedes artificially by crossing the *rapifera* types of *campestris* with different types of *oleracea*.

Thus, by producing swedes it would be possible to transfer genes from different populations of *oleracea* and of turnips to the breeding material of swedes. The genetic variation in the populations of swedes would be increased and new possibilities opened for the breeding of this crop.

Autotetraploid turnips have been produced and bred for about 15 years. This work has given promising practical results, as described by JOSEFSSON (1955). Thus, there was a good material of tetraploid turnips available. For production of artificial rape autotetraploids of a number of different *oleracea* types have been produced earlier. Several new autotetraploids, however, have been obtained. This is especially true of varieties of the kohlrabi type. Their swollen stem is a character which makes kohlrabi types very interesting as parent components in producing swedes.

In the year 1952 crosses were made on a large scale between available tetraploid kale and tetraploid turnips. CALDER (*l. c.*) had pollinated 107 flowers and BECKER (*l. c.*) 487 flowers. No F_1 plants were obtained from these crosses. It was certainly necessary to do these crosses on a large scale, and in the first year about 7500 flowers were pollinated. 1619 seeds were obtained, but nearly all of them were quite like their mothers — maternal inheritance. Only seven plants deviated from the type of the mother. Five of them were F_1 plants — new amphidiploids — in the present paper called A_1 plants, belonging to the A_1 generation. These plants and their progeny were also called a.n.r. 1—a.n.r. 5, *e. g.*, *a*rtificial *n*apus ssp. *r*apifera plant No. 1 *etc.* The other two plants were quite like the father plant. Crosses were made not only between tetraploids but also between diploids, and, owing to a mistake, some crosses were made between tetraploids and diploids. As found in Table 1, which presents some data from the A_1 plants, three of these plants originate from crosses between diploids and tetraploids. Their chromosome numbers, however, are $2n=38$, and they behave like true amphidiploids. Probably unreduced gametes of the diploids have functioned in these crosses. In 1953, about 7500 flowers were pollinated and two (or possibly three) A_1 plants were obtained. They were called a.n.r. 6 and 7. Last year (1954) 6400 flowers were pollinated and with certainty one A_1 plant was obtained. Six possible A_1 plants were also found, and further investigations will show whether they are real A_1 plants. Most of the crosses in 1953 and 1954 were made between kohlrabi and turnips. Altogether 4 paternal plants (*cf.* NISHIYAMA and INAMORI, 1953, pp. 3 and 4) were obtained, and they will be described in another paper.

To provide sufficient seed setting on these A_1 plants, they were

TABLE 1. *Some data from studies on the A_1 generation of artificial swedes.*

Plant No. a. n. r.	Parents	Number of chromosomes	Flower colour	Frequency of good pollen	Mode of fertilization	Seed setting
1	4*n* Yellow Tankard (turnips) × 2*n* Kale	2*n* = 38	dull yellow like swedes	90 %	self-fertile	
2	do.	2*n* = 38	do.	97 %	do.	
3	4*n* Östersundom (turnips) × 2*n* Brussels sprouts	2*n* = 38	do.	98 %	do.	
4	4*n* Yellow Tankard (turnips) × 4*n* F_1 (kale × marrow stem kale)		do.	69 %	self-sterile	
5	do.		do.	70 %	do.	
6	4*n* Silage turnips × 4n Kohlrabi	2*n* = 38	lemon yellow	97 %	do.	10 % (6 pollinated by 7 : 66 %)
7	4*n* »Vege» turnips × 4*n* Kohlrabi	2*n* = 38	dull yellow	96 %	self-fertile	91 %

planted in the fall and the following spring they were flowering and seeds were produced. Thus, they have not been studied as to root formation. Some of the A_1 plants, however, formed swollen roots in spite of this mode of growing. The A_1 plants have been crossed with each other and with the best of our bred stocks of swedes. They have also been crossed with turnips. A.n.r. 1—5 were obtained in 1952, and their meiosis, fertility and morphology could be studied in 1953. The A_2 generation and seeds from the different crosses with the A_1 plants were sown partly in the fall of 1953 for seed production and partly in the spring of 1954 in a root crop trial for studies of root development and type of root as to form and quality (Table 2). A.n.r. 6 and 7 were obtained in 1953 and only the A_1 generation has as yet been studied.

Some data from the A_1 plants are given in Table 1. The chromosome number in the checked plants is as expected $2n=38$. Fertility of the pollen is good with the exception of a.n.r. 4 and 5. Furthermore, these plants are self-sterile as is also a.n.r. 6. The other four A_1 plants are fully self-fertile.

The plants a.n.r. 1, 2, 3, 6, and 7 were very like swedes during all stages of development and could very early be differentiated from plants of the parental types. Their leaves were less hairy than in turnips, and were waxy. All the a.n.r. plants except a.n.r. 6 had a dull yellow flower colour, like the flower colour of the swede. A.n.r. 6 had a lemon yellow flower colour. The sib plants a.n.r. 4 and 5 are less like swedes than the other a.n.r. plants. In earlier stages their leaves were more hairy and waxless — more green — than the swedes. On the other hand, they deviated from the purely matromorphic plants so much that they were selected for that reason. In crosses with a.n.r. 1, 2 and 3 and with natural swedes a.n.r. 4 and 5 have also given quite sufficient seed setting. In crosses with turnips, however, they have only given very few seeds. The plants from these seeds had a very low fertility. Thus, in spite of a somewhat deviating morphology, a.n.r. 4 and 5 have to be regarded as artificial *napus*.

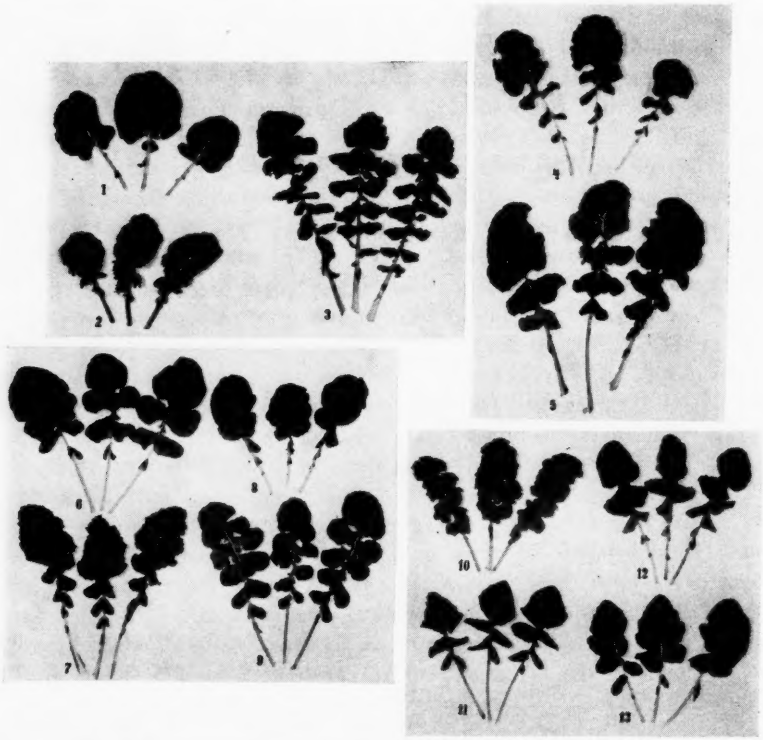
As mentioned above, the plants of the A_2 generation (and plants from other crosses with A_1 plants) were studied partly as seed-producing plants and partly as a root crop. Some data obtained are given in Table 2. The male fertility — frequency of good pollen — is studied in some of the plants and in each combination the highest and lowest values are given to illustrate the variation. The same holds true for the data on seed setting. There is a rather high fertility in the a.n.r. 1, 2 and 3 »families», in crosses between them and in their crosses with the

TABLE 2. *Some data from studies on the A_2 generation of artificial swedes.*

Comb.	Percentage of good pollen	Seed setting per cent	Weight of total pl., g	Weight of root, g	Weight of top, g	Diameter of root, mm.	Leaf stock, mm.
a.n.r. 1×a.n.r. 1	86— 99		449	355	94	68	121
2×2	79—100		743	586	157	92	102
3×3	99—100		534	412	122	87	61
1×2+rec.	82—100	34—79	668	535	133	90	109
1×3+ »	85—100	36—91	681	537	144	89	89
2×3+ »	92—100	48—87	759	592	167	95	85
a.n.r. 4×(1, 2, 3)	34— 95	14—38	744	617	127	87	41
5×(»)	35— 90	38—48	794	633	161	87	61
a.n.r. 1×Bangholm [1]	91— 98	25—45	544	467	77	78	92
1×Victoria	81— 96	31—73	906	747	159	98	74
2×Bangholm	91— 99		924	724	200	104	104
3× »	92—100	66—72	995	740	245	103	97
4+5× »	44— 95	11	729	623	106	88	58
» ×Victoria	52— 89	16	1045	850	195	99	46
Bangholm	97—100		897	711	186	101	94
Victoria	96— 99		786	664	122	101	48

[1] Bangholm and Victoria are Swedish varieties of swedes, *Brassica napus* var. *rapifera.*

The artificial swedes are denominated a.n.r. 1—a.n.r. 5, a.n.r. means *a*rtificial *n*apus var. *r*apifera.

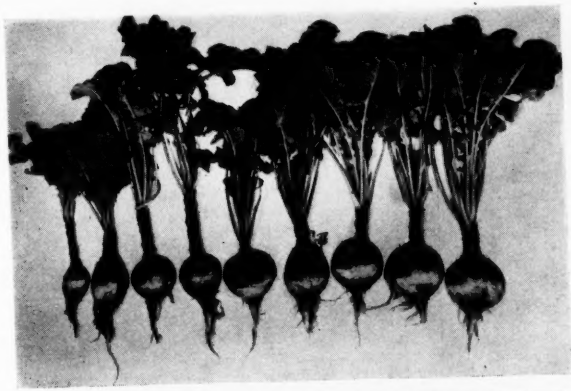


Figs. 1—13. Leaves from artificially produced swedes and from their parental species. 1, Brussels sprouts; 2, kale; 3, turnips; 4—13, different plants of artificial swedes showing a large variation in the type of leaf.

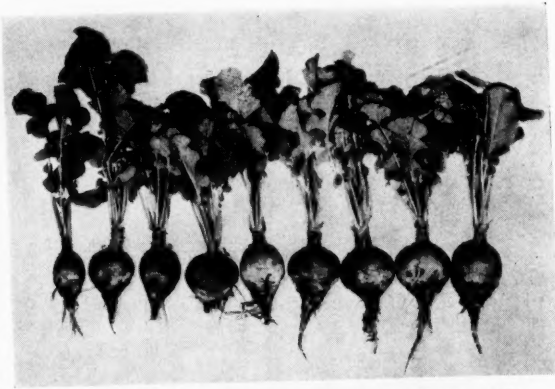
swede varieties Bangholm and Victoria. All cross combinations with a.n.r. 4 and 5, however, result in A_2 plants, most of which are partially sterile.

The variability as to type of leaf is great and some photographs of leaves of the parents *oleracea* and turnips, and of artificial swedes of different types are shown in Figs. 1—13. It is quite clear that all five a.n.r. types from roots more or less like natural swedes. Some types, a.n.r. 1 and a.n.r. 3, are shown in Figs. 15 and 17, while Figs. 14 and 16 show roots of natural swedes, Bangholm and Victoria, respectively. The variation within the different A_2 families is large, and certainly there are plants which look very promising from a farmer's point of

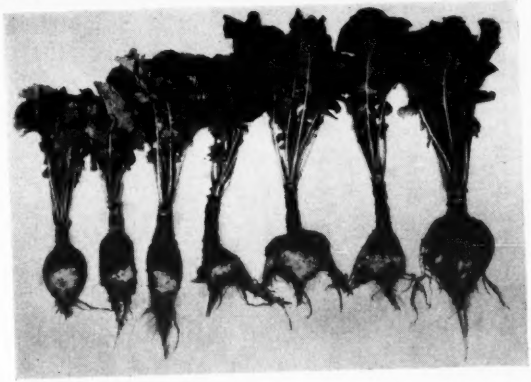
14



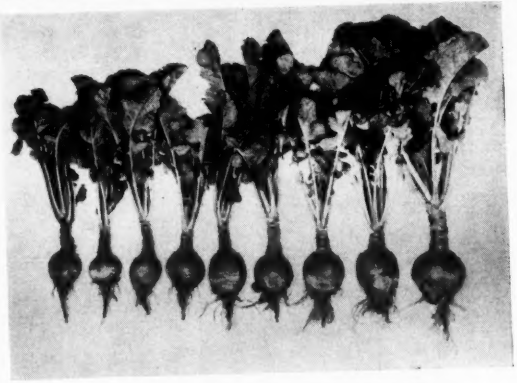
16



15



17



Figs. 14—17. Some plants of natural swedes, Bangholm (14) and Victoria (16) and of artificial swedes, a.n.r. 1 (15) and a.n.r. 3 (17).

view. There are many plants, of course, which have a poor root and these are especially to be found in the family a.n.r. 1. It is interesting to note that there are many different types of roots present, such as cones like beets or turnips, or balls or cylinders. There were types represented which are not to be found in natural swede populations. The means for weight of root, diameter of root, total weight of plant, weight of top, and length of leaf stock are given in Table 2. The means of the artificially produced swede families, which are not selected at all, are somewhat less than those of the high-bred swedes, Bangholm and Victoria, it is true, but this inferiority is not so large as would be expected with regard to the great variation in the artificial material. The most promising result, however, seems to be the high means obtained after crosses between the a.n.r.- plants and the natural swede varieties.

The chemical composition is very much the same as that found in the swede standard material used for comparison. There is a large variation, however, between plants and families in this respect, too.

From this first year of testing the artificially synthesized material of swedes, it is of course not possible to draw any final conclusions as to the value of this material for future breeding. The data obtained, however, inspire the hope of good results in the future and the continuation of the work on this line. The investigations described above have shown that swedes can at least be synthesized by the cross between kale and turnips, and that already in the first A generations there are several plants with large and well-formed roots. There is no doubt that it is possible to transfer genes from the *oleracea* and *campestris* populations to the breeding material of swedes.

The authors wish to express their sincere thanks to Professor ÅKE ÅKERMAN for the great interest with which he has followed this investigation, for valuable comments and stimulating discussions.

The investigation has been facilitated by financial support from the Swedish Agricultural Research Council.

Svalöf in February, 1955.

Literature cited

BECKER, T. 1951. Siebenjährige blütenbiologische Studien an den Cruciferen *Brassica napus* L., *Brassica rapa* L., *Brassica oleracea* L., *Raphanus* L. und *Sinapis* L. — Zschr. für Pflanzenzüchtung, Bd. 29: 222—240.

— 1952. Siebenjährige blütenbiologische Studien an den Cruciferen *Brassica napus* L., *Brassica rapa* L., *Brassica oleracea* L., *Raphanus* L. und *Sinapis* L. II. Teil. — Zschr. für Pflanzenzüchtung, Bd. 31: 72—103.

CALDER, R. A. 1937. Interpollination of *Brassicas*. — The New Zealand Journ. of Agr., Vol. 55: 299—308.

CRANE, M. B. 1943. The origin and relationship of the *Brassica* crops. — Roy. Hort. Soc. Journ., Vol. 68: 172—174.

JOSEFSSON, A. 1955. Tetraploid turnips, a progress in Swedish root crop breeding. — Hereditas 41: 285—287.

KOCH, H. and PETERS, R. 1952/53. Neue Gesichtspunkte der Rapszüchtung. — Wissensch. Zschr. der Martin-Luther-Univ., Halle-Wittenberg, 2: 363—367.

NISHIYAMA, I. and INAMORI, Y. 1953. Polyploidy studies in the *Brassicae*. III. — Memoirs of the Research Institute for Food Science, Kyoto Univ., No. 5: 1—13.

OLSSON, G. 1954. Crosses within the *campestris* group of the genus *Brassica*. — Hereditas, 40: 398—418.

U, N. 1935. Genome-analysis in *Brassica* with special reference to the experimental formation of *B. napus* and peculiar mode of fertilization. — Jap. Journ. Bot., 7: 389—452.

Communications from the Swedish Seed Association No. 90.

OUTLINES OF MEIOSIS IN THE MOSS GENUS SPHAGNUM

By *VEIKKO SORSA*
INSTITUTE OF GENETICS, THE UNIVERSITY, HELSINKI, FINLAND

I. INTRODUCTION

AS is well known, the first cytological studies on the Bryophytes were published in the early days of this century. The chromosome conditions are best known in the orders *Marchantiales* and *Jungermanniales* of the *Hepaticae* and in the variable order *Bryales* of the *Musci* (ALLEN, 1945; VAARAMA, 1950). On the other hand, the more primitive moss orders *Andreaeales* and *Sphagnales* have remained almost entirely unexamined. The order *Sphagnales* includes one family *Sphagnaceae* and this again a single but diversified genus *Sphagnum*. In the literature there is only information regarding the chromosome conditions in *Sphagnum squarrosum* (MELIN, 1915), and later on the chromosome number of this species has been determined by VAARAMA (1953) from a Finnish population. The examinations which I carried out during the years 1952—1954 have revealed the order *Sphagnales* to be in many respects cytologically very interesting.

II. MATERIAL AND METHODS

The material was collected in the neighbourhood of Riihimäki (a market town in South Finland) and includes 27 *Sphagnum* species common there, of which 20 were found in a fertile condition. In the present paper an account is presented of the meiosis of 12 fertile species belonging to the groups *Cuspidata*, *Acutifolia*, *Squarrosa*, and *Compacta*.

The moss individuals from which the specimens were fixed were dried and pressed for verifying the identification of the species. This has very kindly been carried out by Assistant Professor R. TUOMIKOSKI, of Helsinki.

The fixation was performed partly in the field, partly from cultures. As fixatives acetic alcohol (1 : 3) and the fixing solution of Benda, Navashin and Webber were used. The permanent preparations were chiefly made as sections with a paraffin method. Acetic orcein and

aceto-carmine squash preparations were also made of some species. For staining the sections, Feulgen's method was mostly used but also Heidenhain's haematoxylin and crystal violet. In order to make the spindle visible, a combination of Feulgen+haematoxylin staining was used, too (HUGHES-SCHRADER and RIS, 1941). Very good results were obtained by using unbleached Benda fixation, Feulgen staining and phase contrast optics. The section thickness varied from 10—15 μ.

The drawings were made at table level with an Abbe camera lucida, using an oil immersion objective of 100× and ocular of 20×. Thus the magnification is about 3500×.

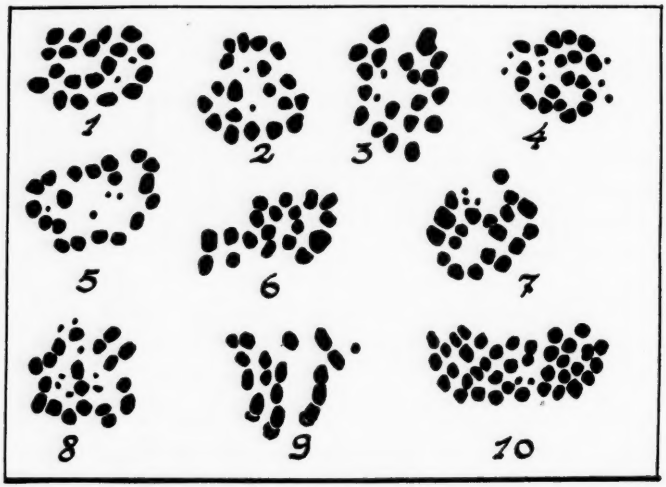
III. OBSERVATIONS

1. Chromosome number and polyploidy

The basic number of chromosomes seems to be the same in all the *Sphagnum* species examined up to now, only the degree of polyploidy being different. In all species there are in addition various numbers of small accessory chromosomes, which often form pairs at first metaphase of meiosis and move actively during the anaphase. In the meiosis of the following species there are 9 bivalents and 1 univalent (figures after the names indicate the number of accessories established in the species in question). (Figs. 1—9.)

S. cuspidatum EHRH. ex HOFFM.	3—4
S. balticum RUSS.	2
S. apiculatum H. LINDB.	2
S. parvifolium WARNST.	4—5
S. fimbriatum WILS.	6—7
S. nemoreum SCOP.	4—9
S. Warnstorfianum DU RIETZ	2—4
S. fuscum (SCHIMP.) KLINGGR.	3—4
S. plumulosum RÖLL.	5—8
S. compactum DC.	2—4

The univalent, which is always transferred to the next generation through the gametophyte of the same sex, probably plays a part in sex determination in *Sphagnum*. Certain phenomena, such as the monoecism of some species, strong secondary pairing in meiosis and somatic pairing in the mitosis of the gametophytes, indicate that the species mentioned above are themselves polyploids. This suggests that in the order



Figs. 1—10. First metaphase of meiosis in *Sphagnum*. Bivalents auto-orientated and completely terminalized chiasmata Feulgen-negative. — Fig. 1. *S. cuspidatum*. — 2. *S. balticum*. — 3. *S. apiculatum*. — 4. *S. fimbriatum*. — 5. *S. nemoreum*. — 6. *S. Warnstorfianum*. — 7. *S. fuscum*. — 8. *S. plumulosum*. — 9. *S. compactum*. — 10. *S. squarrosum*. — 1., 5. and 10. acetic alcohol+Feulgen, 9. Benda+Feulgen, others Navashin+Feulgen. Magnification about 3500×.

Sphagnales there are or have been forms having a still smaller number of chromosomes, maybe 4+1. This number resembles that in certain species of the liverwort order *Anthocerotales* (HEITZ, 1927). Certain structural similarities point to the same order.

The chromosome number of two species investigated:

S. robustum (RUSS.) RÖLL. . . . . (5—11) and
S. squarrosum (PERS.) CROME . . . . . (2— 4)

is double compared with that of the others. In the meiosis of these species there are regularly 19 bivalents and a varying number of the accessories. (Figs. 10 and 28.)

Sporophytes with the triploid number of chromosomes I have never found as yet, indicating that crossing between the species with diploid sporophytes and species with tetraploid sporophytes does not occur, although it seems theoretically possible.

Figs. 11—24. Meiosis in *Sphagnum parvifolium*. — Fig. 11. Early first metaphase, bivalents co-orientated and univalent just leaving the plate. — 12. Polar view of early first metaphase. — 13. Polar view of late first metaphase, bivalents auto-orientated. — 14. Side view of late first metaphase. Univalent moving polewards. — 15. Beginning of first anaphase, sticking between double chromatid groups. — 16. Later first anaphase. — 17. First telophase, elimination chromatin at the site of the metaphase plate. — 18. Interkinesis. — 19. Second prophase. — 20. Second metaphase. — 21.—22. Second anaphase, postreduction. — 23. Second telophase. — 24. Interphase nuclei of spores after meiosis. — Navashin+Feulgen, sections 10 μ. Magnification about 3500×.

2. Meiosis

The spore mother cells grow noticeably before meiosis. During early prophase their chromatophore divides into four daughter chromatophores, which orientate themselves in a mutual position resembling that of the angles of a tetrahedron. During meiosis the size of the mother cells remains unchanged, after the second telophase there follows a rapid phase of growth before the tetrad breaks up into four separate spores.

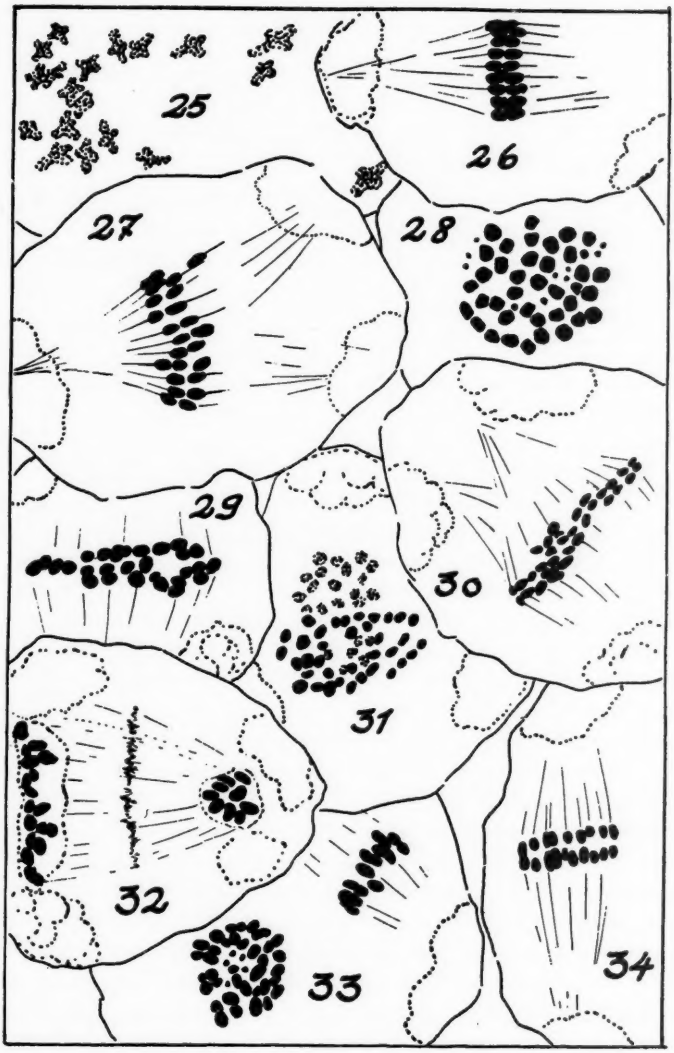
(a) *Chiasma formation.* — In the meiosis of all the *Sphagnum* species investigated, the chiasma is regularly formed only in one arm pair of the bivalent (Fig. 25). Chiasma interference is positive across the centromere region, which is obviously unpaired during diplotene (DARLINGTON, 1937). In the meiosis of certain species (*e. g.*, *S. balticum* and *S. fimbriatum*) the bivalents are found attached themselves in a chain during diakinesis.

(b) *Spindle.* — When the spindle appears at the end of the first prometaphase, its poles are oblong and at right angles to each other like two opposite edges of a tetrahedron. During the first metaphase the spindle becomes quadripolar.

In the meiosis of certain insects, also a somewhat elongated centrosome divides during the first anaphase for the second division, thus turning the spindle into a quadripolar one (STEOPOE, 1930). The second division of meiosis then follows immediately without interkinesis and is equational, which indicates that the centromeres become two-stranded at about the same time as the division of the centrosome occurs, as in the meiosis in *Sphagnum*.

The poles of the spindle, which are still elongated, move close to the chromatophores (Figs. 14 and 27). In some species, like *S. nemoreum* (Fig. 5), a hollow spindle is often found. The quadripolarity of the spindle gives the first metaphase plate a »saddle-like» form (Fig. 29). This form is preserved in the double chromatid groups of first anaphase. On approaching the poles, the groups become longer, following the shape of the spindle (Figs. 16 and 31). The telophase groups and the interkinesis nuclei are often very long and narrow (Figs. 17, 18 and 32). The spindle of the second division of meiosis is a normal bipolar one, but its poles are elongated, too (Figs. 20—23 and 34).

In the spermatogenesis of an earwig two centrioles, which normally form a single centrosome, sometimes begin to separate during the meta-



Figs. 25—34. Meiosis in *Sphagnum robustum*. — Fig. 25. Bivalents during diakinesis. — 26. Co-orientation of bivalents. — 27. Spindle becomes quadripolar and auto-orientation of bivalents starts. — 28. Polar view of late first metaphase. — 29. Side view of first metaphase. The quadripolar spindle bends the metaphase plate »saddle-wise». — 30. Beginning of first anaphase. — 31. Later first anaphase. — 32. First telophase. — 33. Second metaphase, secondary pairing of double chromatids. — 34. Beginning of second anaphase. — Navashin+Feulgen, sections 15 μ. Magnification about 3500×.

phase turning the spindle into a quadripolar one, thus resembling very much the spindle in the sporogenesis in *Sphagnum*, but co-orientation of the chromosome bivalents and prereduction seem to be a rule in earwig (SCHRADER, 1941).

(c) *Co-orientation and auto-orientation.* — During first prometaphase, the chiasmata terminalize so that a clearly Feulgen-negative region appears between the chromosomes forming a bivalent. At first the bivalents are co-orientated (Figs. 11 and 26), but no prereduction takes place, the univalent only occasionally leaving the metaphase plate as early as this phase (Fig. 11). A little later, however, the bivalents become auto-orientated, as in the meiosis of a scale insect, *Lecanium hemisphaericum* (SUOMALAINEN, 1940), obviously as a result of duplication of the centromere region (Figs. 13, 14 and 27—29). The first division of meiosis in *Sphagnum* is equational (Fig. 30). At anaphase the double chromatids separate and go to the poles, resembling the meiosis of the insect order Odonata (OKSALA, 1948).

In the beginning of first anaphase sticking phenomena are often observed (Fig. 15), and between the telophase groups there is so-called elimination chromatin (Figs. 17 and 32). In some cases the shape of the »chromosome pair» which lags behind during first anaphase suggests a certain twist of the bivalent during its auto-orientation followed by prereduction.

The shape and behaviour of the chromosomes differ in a noticeable degree from those cases of auto-orientation in which the centromere structure is diffuse (HUGHES-SCHRADER and RIS, 1941; MALHEIROS, CASTRO e CAMARA, 1947). In the same way visual observation of the spindle suggests the occurrence of a localized kinetochore or at any rate of a multiple kinetochore, which is confined to a narrow region of the chromosome.

The experiments with roentgen irradiation which I carried out last summer (1954) will probably solve the problem of the centromere structure in *Sphagnum*.

The division of the poles of the spindle taking place during the first metaphase of meiosis in *Sphagnum* seems to retard the beginning of first anaphase to the extent that the centromeres find time to double, with the result that co-orientation of the bivalents changes into auto-orientation. The moss order *Sphagnales* thus presents an interesting opportunity for experimental studies of the correlation between spindle action and the division cycle of the chromosomes.

(d) *Postreduction.* — On account of strong secondary pairing the co-orientation of double chromatids is often masked at the beginning of second metaphase. Especially in polyploid species the co-orientation of double chromatids seems to aim at compensation by apparent auto-orientation (Fig. 33). Sometimes also the halves of the same double chromatid seem to be able to pair secondarily with one another (Fig. 20). Only at the end of second metaphase is the co-orientation of the double chromatids evident (Figs. 33 and 34). During second anaphase the breaking of half chiasmata and postreduction follow (OKSALA, 1948). In the chromosome groups of second anaphase the secondary pairing phenomenon may often still be observed.

SUMMARY

Two of 12 *Sphagnum* species investigated, namely *S. robustum* and *S. squarrosum*, have tetraploid sporophytes in contrast to the diploid sporophytes of the others. At first metaphase of their meiosis there are always found 19 bivalents, whereas in the other species there are 9 bivalents and 1 univalent. Besides this, various number of small accessory chromosomes are found in the meiosis of all these species.

The sex determiners are obviously multiple as a result of polyploidy, being located partly in bivalents, partly in a univalent. Many phenomena connected with polyploidy suggest the possible existence of forms having a still smaller number of chromosomes than the species investigated up to now.

The chiasma frequency is very low in *Sphagnum* species. In the beginning of first metaphase the bivalents are co-orientated. At the same time, when the spindle becomes quadripolar, obviously retarding the beginning of first anaphase, the co-orientation of the bivalents changes into auto-orientation, probably because of doubling of the centromeres, and so they are able to divide equationally. The interkinesis nuclei are oblong and situated at right angles to one another, like two opposite edges of a tetrahedron. At the beginning of second metaphase co-orientation of the double chromatids is often uncertain. The second division of meiosis in *Sphagnum* is reductional.

Literature cited

ALLEN, C. E. 1945. The genetics of Bryophytes II. — Bot. Rev. 11: 260—287.
DARLINGTON, C. D. 1937. Recent advances in cytology. — 2nd ed., London.

HEITZ, E. 1927. Über multiple und aberrante Chromosomenzahlen. — Abh. naturw. Ver. Hamburg XXI: 47—57.

HUGHES-SCHRADER, S. and RIS, H. 1941. The diffuse spindle attachment of Coccids, verified by the mitotic behavior of induced chromosome fragments. — J. Exp. Zool. 87: 429—456.

MALHEIROS, N., CASTRO, D. DE e CAMARA, A. 1947. Chromosomas sem sentrómero localizado o caso da *Luzula purpurea* LINK. — Agronomia Lusitana XIV, Tomo II: 95—99.

MELIN, E. 1915. Die Sporogenese von *Sphagnum squarrosum* (PERS.), nebst einigen Bemerkungen über das Antheridium von *Sphagnum acutifolium* (EHRH.). — Svensk Bot. Tidskr. 9: 261—293.

OKSALA, T. 1948. The concept and mechanics of chromosome reduction. — Hereditas XXXIV: 104—112.

SCHRADER, F. 1941. The spermatogenesis of the earwig *Anisolabis maritima* BON. with reference to the mechanism of chromosomal movement. — J. Morph. 68: 123—148.

STEOPOE, I. 1930. La spermatogénèse chez *Nepa cinerea*. — Ann. scient. de l'Univ. Jassy XVI: 611—654.

SUOMALAINEN, E. 1940. Beiträge zur Zytologie der parthenogenetischen Insecten. II. *Lecanium hemisphaericum* (Coccidae). — Ann. Acad. Scient. Fenn., Ser. A., LVII, No. 6: 1—30.

VAARAMA, A. 1950. Studies on chromosome numbers and certain meiotic features of several Finnish moss species. — Bot. Not. 1950: 239—256.

— 1953. Some chromosome numbers of Californian and Finnish moss species. — The Bryologist 56: 169—177.

STUDIES ON X-RAY INDUCED VIABILITY MUTATIONS IN THE THIRD CHROMOSOME OF DROSOPHILA MELANOGASTER

By *RAPHAEL FALK*

INSTITUTE OF GENETICS, UNIVERSITY OF STOCKHOLM

I. INTRODUCTION

WHEN MULLER (1928) published his early work on the induction of mutations by X-rays, he pointed out that there were indications that the number of mutations with small effects is much higher than of those which are commonly used in daily laboratory work. He emphasized that the group of detrimentals should be much more abundant than the lethals. A short time later some attempts were made to determine the frequency and intensity of those detrimental mutations. The works of TIMOFÉEFF-RESSOVSKY (1934, 1935) and KERKIS (MULLER, 1934; KERKIS, 1938) showed surprising agreement in their results, in that the detrimentals were 2—3 times more abundant than the lethals. Though work on subvitals has been undertaken since then, particularly as regards their importance in natural populations (DOBZHANSKY, HOLZ and SPASSKY, 1942; DOBZHANSKY and SPASSKY, 1944; PAVAN *et al.*, 1951; DOBZHANSKY and SPASSKY, 1953; WALLACE and MADDEN, 1953; DOBZHANSKY and SPASSKY, 1954), only in the last few years has an interest in their nature been renewed (MULLER, 1950; STERN *et al.*, 1952; KÄFER, 1953).

The present work was undertaken in order to determine the frequency and intensity of detrimental mutations in one of the X-rayed autosomes of *Drosophila melanogaster* and, at the same time, to try to determine their degree of dominance. The experiments were planned so as to work with flies which were isogenic except with regard to X-ray induced mutations in their third chromosome. As a measure of vitality it was planned to use the number of eggs which a female of a certain genotype laid, and the hatchability of the eggs of that genotype.

II. EXPERIMENTAL

1. Material

In order to produce isogenic flies containing irradiated third chromosomes and an unirradiated set of the other chromosomes, the following procedure was adopted.

A single female of the Oregon-R stock was crossed to $sc^{S1}B$ InS sc^{8}; $al^{2}Cy$ lt^{3} sp^{2}/dp b Pm^{1}; ru h D^{3} InCXF ca/Sb In3R. This genome will be referred to as M5x; Cy/Pm; D/Sb; the M5x chromosome being a Muller-5 chromosome without the w^{a} mutant, thus permitting a distinction between Pm and non-Pm flies. From this the following three genotypes were produced:

(1) M5x; Cy/Pm; Sb/+
(2) +/+; +/+; Sb/D
(3) +/+; +/+; +/+

The wild type chromosomes were made isogenic, through a modification of MULLER's system for the construction of homozygous stocks (MULLER, 1936).

Strains of these three genotypes will be referred to as »strain 1», »strain 2» and »control stock».

Males 2—3 days old of strain 1 were irradiated with 2000 roentgen units. This age was chosen in order to insure uniform sensitivity to irradiation (LÜNING, 1952). They were immediately crossed to females of strain 2, 3—4 pairs per vial. Single virgin females of each of the four possible genotypic combinations among the offspring were crossed to 3—4 males of the strain 2. Each such female carried only one irradiated third chromosome from the isogenic stock. All her other chromosomes carried either markers (irradiated or non-irradiated) or +genes unirradiated and isogenic already introduced from strain 2. From the progenies of these single females an attempt was made to select flies with as few markers as possible and then to remove the remaining markers through repeated brother sister crosses (Fig. 1).

The fourth chromosome was not controlled at all, and could thus cause heterogeneity, particularly as an irradiated fourth chromosome could be introduced into the experimental stock in one or two doses. Tests showed that the SbIn3R chromosome used for isogenisation had no inversion in the left arm and crossing over in this arm was free or even enhanced. Repeated genetic tests showed that the $al^{2}Cy$ lt^{3} sp^{2} chromosome contained inversions in both arms, but because of the absence

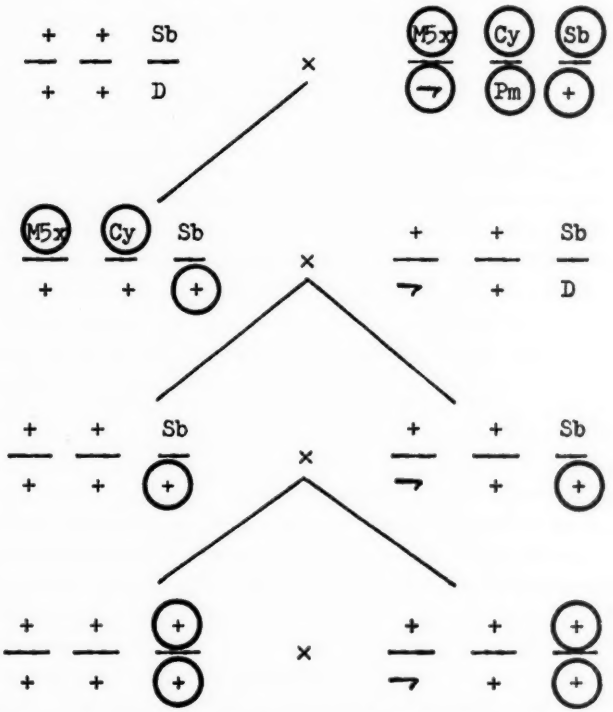


Fig. 1. Diagram of the crosses made in order to isogenize the third irradiated chromosome and to substitute all the others by non-irradiated isogenic ones. The irradiated chromosomes are indicated by a circle.

of the L marker in the right arm, it is possible that while preparing the stocks some undetected crossing over occurred near the centromere. All these factors could, and possibly did, introduce a certain degree of heterogeneity in the material.

The word »experimental stock» will be used for the progeny of flies of the type shown at the bottom of Fig. 1 coming from a single female with an irradiated third chromosome (line 2 of Fig. 1). Different experimental stocks thus carry irradiated third chromosomes originating from different spermatozoa of the irradiated males (line 1, Fig. 1).

Those experimental stocks which produced only flies heterozygous for the third chromosome were crossed for a further one or two generations, and if they still did not give offspring homozygous for this

chromosome they were classified as lethals. Some experimental stocks which gave very few homozygous offspring were lost through these attempts to produce homozygosis. They apparently had a very low viability presumably caused by an irradiation induced mutation in their third chromosome.

The first irradiation was set up as a pilot experiment without recording the loss during isogenisation. From this experiment, 5 experimental stocks were tested. The later irradiation was made with about 150 males of strain 1, which were crossed to females of strain 2 in 40 vials. Of their progenies 180 virgin females were crossed individually and perpetuated as described.

From the 180 females, 92 experimental stocks gave at least one homozygous fly and were classified as non-lethals. A further 18 stocks where there appeared repeatedly only heterozygous flies were classified as lethals. The remaining 70 were lost during isogenisation. Of the 92 non-lethal experimental stocks, 27 were lost during attempts to propagate and transfer from vials to bottles. Of the remaining 65 experimental stocks which were transferred and kept in bottles, only 47 were tested, while the remaining died or were lost before they could be tested. Of the 18 lethals only 8 were maintained (Table 1).

TABLE 1.

	Non-lethals				Lethals			Total
Lost during the process of isogenisation	Analysed	Lost during propagation and transfer from vials to bottles	Lost in bottles before final analysis	Total	Maintained	Lost	Total	
70	47	27	18	92	8	10	18	180

The loss of experimental stocks was very great. Of 180 only 55 (47 non-lethals and 8 lethals) could be maintained. A portion of them probably died because of low viability. For example, some experimental stocks in which only 1—2 wild type flies were produced were in all probability semi-lethals. However, many of them it seems, died because of non-genetic factors and because of the detrimental effect of the other irradiated marked chromosomes prior to their substitution by isogenic non-irradiated chromosomes. The maximum time that an experimental stock was kept in culture bottle before testing could be done was not more than 5—6 months, or about 10—12 generations.

2. Analysis of viability

Initially two characters were chosen as criteria of viability: the number of eggs laid by a female, and the hatchability of those eggs. It was soon apparent, however, that the number of eggs is so dependent on environmental factors that the test was discarded. Hatchability was therefore the main criterion and was measured in the homozygous as well as in the heterozygous condition.

The following three types of »crosses» were made.

(a) Matings within the control stock.
(b) Mating within the different experimental stocks.
(c) Mating between control stock and the different experimental stocks or *vice versa.*

From the bottles in which the experimental stocks were kept, flies were transferred to 5 vials, 4—5 pairs per vial, in which was placed a piece of cardboard with a cube of food consisting of maize meal, agar and syrup and sprinkled with a little dry yeast. The flies were allowed to lay eggs for three days and then discarded, while the food cube with the larvae was put in a vial with absorbant paper soaked in a rich yeast suspension. After seven days, when flies began to hatch, as great a number as possible of virgin females and of males was collected and kept separately until the third day after eclosion when they were paired, one female and one male per vial. Of each cross 20 vials were set up. In each vial a cube of food containing 2 % agar, a little syrup and a little charcoal was added on a piece of cardboard, and the flies allowed to lay eggs for three days. A check was then made under a dissecting microscope in order to determine which vials contained larvae. This was made to insure that the non-hatching of eggs should not be due to non-fertilization. The test will be called *insemination test* and will be said to be positive when larvae were present. The first 5 vials (later the first 8) with a positive insemination test were given a record number and fresh cubes of blackened food were added. This was repeated 4 more times at 24 ± 2 hours intervals. The vials (without flies) were kept for a further 28 hours when counts were made of the total number of eggs and of the number which had hatched. Thus results were obtained from the fifth to the tenth day of age of the females.

According to STRAUS (1942) and GOWEN (1952), these are the days of maximum egglaying. During the food shifting it was noted when flies died. In those cases where a female died, the vial was discarded, but

when the male died counts were made as usual, the assumption being that the sperm in the female was sufficient for at least five days (KAUFMANN and DEMEREC, 1942).

The crosses were made in time groups separated by about a week's interval. Altogether 12 such time groups were made. Each group included the three »crosses» mentioned above (p. 263). Cross a, control stock; in one of the 12 time groups the control failed. Cross b, crosses within experimental stocks, 5 to 8 different experimental stocks per time group. Cross c, crosses between the same experimental stocks and the control stock. 20 vials were set up for each different cross, 5—8 of which were analysed as described. In this way it was possible to obtain the hatchability and the number of eggs of the homozygotes and the hatchability of the heterozygotes.

The statistical analysis was based on the number of eggs in only those vials where the female laid eggs for 5 consecutive days. It was planned to use 5 as the minimum number of females of each experimental stock to be analysed. Later, however, although an initial number of 8 females was taken, there were still experimental stocks where only 4 or even 3 females were left for analysis.

III. STATISTICAL

The percentages of hatchability are calculated from the number of eggs per female. These numbers vary from 30 to 427 eggs, the average being 146 eggs per female. This suggests a possible need of weighting the percentages. COCHRAN (1943) showed that when the binomial variance is less than 30 % of the total variance, equal weighting, *i. e.*, independence of the number from which the percentages were calculated, is sufficient. In order to estimate the percentage of binomial variance he gives the following formulae.

$$\text{Binomial variance: } \bar{f}(1-\bar{f})/\bar{n}_h$$
$$\text{Extraneous variance: } s^2_w - \bar{f}(1-\bar{f})\bar{n}$$

where f is the average proportion of the eggs hatched, $\bar{n}_h$ the harmonic mean of the number of eggs laid per female, $\bar{n}$ the average number of eggs laid per female and s^2_w the variance within matings.

Pooling the figures of the whole experiment the following proportions were found

Binomial variance 5,7 %
Extraneous variance 94,3 %

Thus as an average of only about 6 % of the variance is binomial equal weighting can be adopted, and therefore each percentage can be used directly without considering the number of eggs from which the percentage was calculated.

The observed variance, s^2_{obs} of the different experimental stock is composed of three parts, sampling variance s^2_s, environmental variance s^2_e, and genotypic variance s^2_g. As the control stock is looked upon as isogenic, its observed variance is composed of only the first two parts: $s^2_{obs}=s^2_s+s^2_e$. Within each of the time groups, egg counts were made on a number of consecutive days. If p_i denotes the proportion of hatched eggs in the control stock and $q_i=1-p_i$ and if n_i the corresponding number of eggs, the sampling variance within this i:th culture is $\frac{p_i q_i}{n_i}$. The all over sampling variance can then be estimated as $s^2_s=\frac{\bar{p}\bar{q}}{N}\sum\frac{1}{n_i}$ (WALLACE and MADDEN, 1953), where $\bar{p}$ and $\bar{q}$ are the averages of p_i and p_i, and N is the total number of vials (=females). As described earlier there were 12 time groups, but the control stock failed in one of these. The total number of vials from which hatching percentages of the normal stock could be computed was N=70. Excluding from the counts all vials in which the females laid less than 50 eggs, the totals of the control stock were:

eggs laid (Σn_i)	10312
eggs hatched	9459
number of vials (N)	67
$\sum\frac{1}{n_i}$	0,49735

$$\bar{p}=\frac{9459}{10312}=0{,}9173$$

$$\bar{q}=1-\bar{p}=0{,}0827$$

$$s^2_s=\frac{(0{,}9173)(0{,}0827)}{67}(0{,}49735)=5{,}63$$

The analysis of variance is shown in Table 2.

These figures give the estimated variance due to environment as

$$s^2_e=9{,}14-5{,}63=3{,}51$$

The t-test for the various experimental stocks in the homozygous and heterozygous condition is then:

$$t = \frac{q_c - q_r}{\sqrt{\frac{p_c q_c}{n_c} + \frac{3{,}5}{k_c} + \frac{p_r q_r}{n_r} + \frac{3{,}5}{k_r}}}$$

where q_c is the hatchability of the control stock and q_r the hatchability of an experimental stock tested simultaneously, $p_c = 1 - q_c$, $p_r = 1 - q_r$, n_c is the number of eggs laid in the control stock, n_r the number of eggs laid in the experimental stock, k_c the number of vials of the control stock and k_r the number of vials of the experimental stock. A probability level of 0,01 was taken as indicating significant deviation from the control stock of the simultaneously tested experimental stock.

TABLE 2. *Analysis of variance of control stock tested on 11 different occasions (time groups).*

	df	Sum of squares	Mean square
Between time groups	10	1629,90	162,99
Within time groups	56	511,85	$9{,}14 = s^2_{obs}$
Total	66	2141,75	

IV. RESULTS

The results of all experiments are shown in Table 3. Although this table can give no picture of the way in which the irradiation influenced genes responsible for hatchability act, it shows that on the average the treatment reduced the hatchability by 8 % compared with the normal, and further that at least part of the mutations were partially or completely dominant.

1. Control stock

Tests of hatchability were made in 12 time groups in 11 of which there were successful controls. Table 4 gives the distribution of the hatchability of the control stock in these 11 time groups. As can be seen 10 of these tests are concentrated about the mean of 92,2 % hatchability, but the difference in hatchability between the time groups is highly significant ($F = 17{,}83$; $P < 0{,}001$).

The control stock was synthesised so as to be isogenic. If however, in spite of the isogenisation, it included some genetic variability, a possible but unintentional selection of the more vital types may have been in-

TABLE 3. *Summary of the results from all experimental stocks tested.*

	Number eggs hatched	Total number eggs laid	Average hatchability per cent
Control stock	9550	10427	91,6
Experimental stocks			
Homozygotes	36510	43424	84,1
Heterozygotes	42724	48524	88,1

TABLE 4. *Distribution of hatchability of the control stock in 11 time groups. Average hatchability (all time groups given equal weights) 92,2 %.*

Hatchability %	78	80	82	84	86	88	90	92	94	96	98
frequency		1				1	2	2	4	1	

troduced from two different sources. The results of the insemination tests varied very much from time group to time group. In one, no vial contained larvae at the insemination test, and in this the control stock accordingly failed. In some of the time groups all vials contained larvae at the insemination test, but on the average only 54 % of the vials gave a positive insemination test. If lack of larvae; *i. e.*, a negative insemination test, was due to a lower viability of the mated pair, its exclusion from the experiment thus introduced genetic selection. Another possible source of selection for the more viable flies was that those vials in which females had died during the 5 days of egglaying were excluded from the calculations. About 11 % of the flies of the control stock which began the hatchability test died before these five testing days had elapsed, which means that 3 % of the vials were excluded. These possible sources of selection will be discussed later.

2. Experimental stocks

Within each time group the experimental stocks, homozygous and heterozygous, were compared with the simultaneously tested control stock; and a comparison was also made between the homozygotes and the heterozygotes of the same experimental stocks. In the single time group where no simultaneous test of the control was available the mean of the other 11 time group was used. In the one time group where the control stock's hatchability was unusually low (see Table 4) some of the

TABLE 5. *Description of the method by which the experimental stocks were classified as belonging to different types with regard to their hatchabilities (C=control stock, E hom=experimental stock in homozygous condition, E het=experimental stock in heterozygous condition, s=significant, ns=non-significant).*

Comparison made			Classification
C–E hom	C–E het	E hom–E het	
ns	ns	ns	Normal hatchability
			Reduced hatchability
s	s	ns	Dominant
s	s	s	Partially dominant
s	ns	s	Recessive

TABLE 6. *Distribution of the hatchabilities of the irradiated experimental stocks. A number of the experimental stocks was tested more than once (see Table 7). The present table, however, shows only results of one of the tests. The cases in which two tests of any experimental stock did not agree are dealt with in detail in Tables 7 and 8 and in the text. For the one case left out — see text.*

hatchability	% of homozygote	55	60	65	70	75	80	85	90	95	Total
normal								1	7	7	15
reduced hatchability	dominant	1		1	1	2	2		1		8
	partially dom.	1		1	1			1			4
	recessive			1	3	2	5	7	1		19
Total		2		3	5	4	7	9	9	7	46

comparisons were made both with the mean of the 11 time groups and with the simultaneous control stock.

The comparisons were, as described above, made by a t-test. According to whether the differences were significant, stocks were classified as belonging to one or another of the types shown in Table 5. The classification of the analysed experimental stocks is shown in Table 6 where the hatchabilities of the homozygotes are also to be found. The table includes, however, only 46 of the 47 analysed experimental stocks, the one not included being labeled with the letter e in Table 7 and presented with more detail in Table 9.

TABLE 7. *Distribution of hatchabilities of experimental stocks which were tested twice. The numbers in brackets are estimates from tests in which there was only one vial left for the hatchability test and therefore were excluded from the detailed calculations. The letters refer to explanations in the text.*

First test / Second test		Normal hatchability	Reduced hatchability			Total
			Dominant	Partial dom.	Recessive	
normal hatchability		1 + (3)	1 (a)	1 + (1) (b) (c)	2 (d)	9
reduced hatchability	dominant		4		1 (e)	5
	partial dom.			1 + (1)		2
	recessive	1 + (1) (f) (g)			6	8
Total		6	5	4	9	24

A second test of an experimental stock was made when, in the insemination test, too few vials with larvae were available to ensure a reliable hatchability test; or when during the hatchability test itself so many females died that the results were insufficient to be included in the final evaluation. With some further experimental stocks a second test was made merely to study the repeatability of the results (Table 7). In 18 experimental stocks there were in both tests sufficient vials for full analysis. In 6 of the experimental stocks one of the tests included only one vial. Table 7 shows how the hatchabilities changed from the first to the second test. The figures in brackets correspond to the 6 experimental stocks with only one vial for one of the tests.

If the cases in brackets are included, it is seen that 16 out of 24, or $^2/_3$ of the experimental stocks reacted similarly in the two tests. 8 of the experimental stocks given special letters in Table 7 changed their classification from the first to the second test (Table 8). The question is whether this was due to selection or to environment. A more detailed analysis of these cases will therefore be made.

As pointed out earlier, if the experimental stocks were not isogenic selection may act at three different stages: (1) during the time between the two tests when they were kept in culture bottles; (2) during in-

TABLE 8. *Details concerning the 8 experimental stocks from Table 7 which reacted differently at the two tests. The table shows, with regard to insemination test, the number of vials with positive test and the total number of vials; with regard to fly mortality, number of flies that died before the 5th day of egglaying and the total number of flies. The sign x together with the test number indicates which of the two tests was used for Table 6.*

Test No.	Insemination test		Mortality		Hatchability %	
	Homoz.	Heteroz.	Homoz.	Heteroz.	Homoz.	Heteroz.
a 1x	2/18	5/19	1/2	1/6		67
a 2	10/19	11/20	4/8	0/16	93	89
b 1x	2/15	10/20	0/4	0/5	56	79
b 2	11/34	12/21	2/14	1/16	89	89
c 1x	2/20	13/19	0/2	1/14	75	89
c 2	3/39		0/6		81	
d_1 1x	13/28	15/18	0/16	0/8	78	92
d_1 2	3/16	11/26	2/16	2/16	92	95
d_2 1x	11/18	13/20	1/16	0/16	81	96
d_2 2	12/28	14/20	6/16	1/16	93	93
e 1	4/15	8/18	0/4	1/16	42	92
e 2	9/29	10/18	0/10	1/16	72	76
f 1	14/25	7/13	1/8	1/14	94	95
f 2x	19/38		0/14		89	
g 1	4/19	13/20	1/6	0/16	94	91
g 2x	8/30		1/8		90	

semination test if the proportion of vials with negative test was high; (3) during the experiment itself through mortality among the egglaying females. From Table 8 it is thus concluded:

Experimental stocks a and b: The first test showed few vials with positive insemination test, and low hatchability. The probable cause was selection within the culture bottles between the two tests.

Experimental stock d: The proportion of vials with positive insemination test decreased from the first to the second test, and in one of these stocks the mortality increased. The probable cause was selection in the second test during insemination test and among egglaying females.

Experimental stock e: No change in insemination test or in female

mortality. The probable cause was different reactions to changes in environment (see also Table 9).

Experimental stocks c, f, g: No change in insemination test or in female mortality. As in one of the tests there was a significant deviation from normal in hatchability it is concluded that this reduced hatchability is the correct value.

From this survey of the experimental stocks with differing results in the two tests, it can be concluded that selection within the culture bottles before the second test for hatchability was made, had not too great an influence. The other two factors — differences in insemination tests and a possible selection of females of higher viability during the experiment itself — may introduce a greater source of error. It should however be stressed that all three factors influence the results in the direction of masking the effects of the irradiation on the hatchability. It appears that interaction between genotype and environment on hatchability is not too high, which justifies the estimation of the different parts of the variance without including such an interaction.

Of the 47 experimental stocks analysed, 24 were tested on two different occasions and the remaining 23 were tested only once. 20 of the latter had a sufficient number of vials with positive insemination test (which of course was one of the reasons why they were not retested). There were, however, 3 experimental stocks with a low number of vials with positive insemination test, which were tested for hatchability only once.

(1) One of these had a hatchability, which was significantly higher than the control stock. It was in Table 6 classified as having normal hatchability.

(2) One experimental stock classified as a full recessive in Table 6 had in the heterozygous condition a low number of vials with positive insemination test — if there was a selection through the insemination test the experimental stock should better fit into the class of partial dominance in Table 6.

(3) The third case was a fully dominant reduction to a hatchability of 90 %. If there was selection during insemination test this experimental stock should fit better a little more to the left in Table 6.

A special case is represented by one experimental stock (e in Tables 7 and 8) which at one occasion showed a very low hatchability of the homozygote whereas the heterozygote had normal hatchability — which means a complete recessivity. — When this experimental stock was retested the heterozygote showed about the same reduction as the

TABLE 9. *The change in hatchability and dominance for one experimental stock tested twice.*

	Hatchability	
	Homozygote	Heterozygote
First test (time group 1)	41,9 %	90,3 %
Second test (time group 6)	72,4 %	75,5 %

homozygote — a full dominance, but the reduction from normal hatchability was less (Table 9).

V. CONCLUSIONS AND DISCUSSION

As already mentioned, the loss during isogenisation of several experimental stocks was probably caused by their being semilethal (viability less than 50 %). As can be seen (Table 6), no such semilethal experimental stock was left for hatchability test. The present work is therefore confined to *subvital mutations*. The subvitals show a corresponding decrease in frequency with lowering in viability. This is in good agreement with other works on the frequencies of subvitals (TIMOFÉEFF-RESSOVSKY, 1934, 1935; KERKIS, 1938; MULLER, 1950, but see KÄFER, 1953). MULLER (1950) expected on theoretical grounds, that the degree of dominance should increase the less the reduction from the normal is in the homozygote. This can only partly be confirmed by the present work. Recessives are much more frequent than fully or partial dominants. There is no tendency of increasing frequency of dominant experimental stocks in the right part of Table 6, neither is there a decrease of recessive experimental stocks in the left part of Table 6. This could, of course, be due to the limited material (31 experimental stocks), but perhaps even more by masking of the results because of the high variability between the vials of one and the same experimental stock. If it is so, it may be thought that some of the experimental stocks which in Table 6 are recorded as normals, should more correctly be included among dominants with slightly reduced hatchability. In the same way perhaps some of the stocks recorded as fully recessives are slightly dominant. On the other hand, those 4 stocks which had a significant partial dominance (Table 6) show the relation between the degree of dominance and the reduction of hatchability, as expected by MULLER (Table 10).

The abundant presence of subvital mutations has already been shown

TABLE 10. *The relation between the degree of reduction of hatchability in the homozygote and the degree of dominance expressed as % reduction of heterozygote/homozygote in the stocks showing significant partial dominance.*

Difference of hatchability % between control stock and experimental stock when		% reduction hetero/homo
Homozygote	Heterozygote	
6,6	5,4	82
24,3	11,2	46
26,8	10,6	40
34,8	11,9	34

by TIMOFÉEFF-RESSOVSKY (1934, 1935) and KERKIS (MULLER, 1934; KERKIS, 1938). Both made their investigations on irradiated X-chromosomes and used the proportion of females heterozygous for the irradiated X-chromosome to males with this chromosome, as a measure of viability. By the very nature of their experiments they were neither able to eliminate the influence of the irradiated autosomes which were uncontrolled in the flies counted, nor the influence of dominance (partial or complete) of these detrimental mutations.

Considerable proof has already accumulated as to the incomplete recessivity of many genes, particularly of those which are lethal in homozygous condition (MASING, 1938, 1939; GUSTAFSSON, 1946, 1947, 1952; MEYER *et al.*, 1949; WALLACE, 1950; STERN *et al.*, 1952; WALLACE and MADDEN, 1953; and others). The reasoning that the X-chromosome has a lower rate of mutation, being a compensation system better buffered against a stronger degree of selection (BERG, 1937 a, 1937 b; MULLER, 1943, 1950, but see KÄFER, 1953) makes it doubtful if, and to what extent, one can estimate the frequency and intensity of such mutations in the whole genome by extrapolation of results from the X-chromosome. Although DOBZHANSKY and his colleagues (DOBZHANSKY, HOLZ and SPASSKY, 1942; DOBZHANSKY and SPASSKY, 1944; PAVAN *et al.*, 1951; DOBZHANSKY and SPASSKY, 1953; WALLACE and MADDEN, 1953; DOBZHANSKY and SPASSKY, 1954) have determined the subvitality of genes located in the autosomes, the remainder of the genome was uncontrolled. Their comparison of flies heterozygous for the tested chromosome with those homozygous for it demands that all the characters tested should always be completely recessive. That this is not the case can no longer be doubted.

At least part of the unexpected results of KÄFER (1953) of the numbers

of second chromosome subvitals may be explained if it is remembered that many subvital mutations have a certain degree of dominance. These mutations will reduce the number of the heterozygotes as well as the number of the homozygotes, so that in a viability test made by comparing heterozygotes with homozygotes they are classified as normals or nearly so. That this factor played indeed a part in her work can be seen by the high number of cultures with a low number of heterozygous flies (her Table 3).

All these facts as well as the growing interest in such subvital mutations in natural populations (cited above) and their possible influence on the wellbeing of humanity (MULLER, 1950) makes it desirable to know more about their nature and mode of action. An analysis of viability based on the numbers of eggs laid by flies of a definite, controlled genotype, and the hatchability of eggs of this genotype, would enable us to overcome most of the above mentioned difficulties. The effect of the genotype on the capacity for egglaying was shown by STRAUS (1942) and GOWEN (1952), and they further showed that such genes are scattered over all of the chromosomes of *Drosophila melanogaster* in proportion to their length.

Throughout the descriptive part of the present paper, attention has repeatedly been drawn to the great part of uncontrolled factors in the experiments. The hatchability itself appears to be rather constant in different environments, though the case of change of reaction shown in Table 9 should be kept in mind. On the other hand, the number of females of the present investigation which laid fertilized eggs in the first four days of life (or perhaps, in the first 1—2 days of the presence of males), observed by the insemination test, seems to vary considerably. It appears that other factors, such as crowding of the flies before they are mated, produce a great part of the high variability within, as well as between tests. While these factors could act as selective agents only by unintentional choice of the more viable flies during the final analysis, there could also be selection or genetic drift during the time the experimental stocks were kept in bottles prior to the final analysis (especially as just the weak experimental stocks had fewer flies when transferred to new culture bottles). As shown in the above analysis of experimental stocks which were tested more than once the selection within the bottles before the tests were made, had not affected the results seriously.

A possible source of genetic variability is accumulation of new mutations in the experimental stocks during the process of isogenisation

and while remaining in culture bottles until tested for hatchability of the eggs. The process of isogenisation gave further some reason to suspect full isogeneity, thus possibly supplying the variability on which the selection works.

Of the 110 isogenised chromosomes of the present study, 92 were non-lethals and 18 lethals as indicated before. The proportion lethals : non-lethals is thus 18 : 92. Of these 92 only 47 were left to be analysed and therefore the corresponding number of lethals could be estimated as $18 \times 47/92 = 9{,}2$. Taking all the chromosomes which were affected by irradiation, it is found that 32 (31 from Table 6 and the experimental stock which was excluded from the table and mentioned in Table 9) showed a reduction of viability as measured by hatchability, compared with the estimated number of 9,2 of completely lethals. This indicates that the subvital mutations are about 3,5 times more common than the lethals. This value of 3,5 is higher than that found by TIMOFÉEFF-RESSOVSKY (1935) and KERKIS (1938) who got a factor of 2—3. As the estimations of subvitals in the present experiment must give minimum values it seems to support MULLER's (1950) calculations of a factor of 5. In the present study as well as in those of TIMOFÉEFF-RESSOVSKY and KERKIS the reduction of viability is caused by the mutations themselves, but in the studies of natural populations (DOBZHANSKY, HOLZ and SPASSKY, 1942; PAVAN *et al.*, 1951; DOBZHANSKY and SPASSKY, 1953, 1954; and others) the distribution of these reductions is due to selection *and* mutation. If the frequencies of the chromosomes are plotted against their degree of detriment one gets a two peaked curve, one peak for the full lethals and one in the opposite end for the weak subvitals. Two hypotheses are suggested as an explanation of this phenomenon: (1) As selection works mainly on the heterozygote, the group of the detrimentals which are in the part of the curve between the lethals and the weak subvitals were much more selected against than the lethals during the phylogeny and are therefore less often produced (*cf.* BERG, 1937 a). (2) Another hypothesis is that the lethals belong to another physiological group than the subvitals (*cf.* BEADLE, 1951) and the two-peaked curve is therefore a result of two curves imposed on each other.

The very important problem of the relation of the degree of dominance to the degree of subvitality can only be dealt with partly. Table 10 shows that the less subvital mutations show a higher degree of dominance and are therefore more, or at least as effectively, selected against as the stronger subvitals — which are completely recessive

(MULLER, 1950). Nothing can be said how these two factors are correlated, no such relation can be seen in Table 6, but this can be a masking of the real condition. More evidence is therefore needed in this point.

Of 56 chromosomes irradiated (47 tested+an estimate of 9,2 lethals) only a maximum of 15 chromosomes were not affected by the irradiation. Thus at least in those characters tested by this experiment, the effect on the other 73 % was a partial or complete reduction in viability. This again points to the frequency of such mutations and emphasises the importance of understanding their behaviour. It should be noted that the high hatchability of the control stock excludes practically any possibility to detect supervital mutations.

ACKNOWLEDGEMENT

I wish to express my sincere thanks to Professor GERT BONNIER who suggested the course of study on a problem which he himself had carried out the initial experiments and who has constantly advised me on that work and on the preparation of this paper.

I am also indebted to Professor C. P. SWANSON and Miss M. E. MACKENDRICK for revising the English text.

SUMMARY

(1) Through a special crossing scheme it was possible to produce isogenic experimental stocks in which the third chromosome alone was irradiated (the fourth chromosome was neglected).

(2) During the production of these experimental stocks a great number was lost before they could be tested. This was partly due to non-genetic causes. However, several stocks were so weakened by the X-ray induced third chromosome mutations, that they were lost by this cause. The present work is therefore mainly confined to the subvital mutations.

(3) 47 experimental stocks, the third chromosome of which was irradiated with 2000 r were tested. As a measure of viability the egg hatchability was used. Hatchability was determined in homozygous and heterozygous condition.

(4) Of each experimental stock the hatchability of eggs laid by 3—8 females during 5 days was determined. The variability within experimental stocks was great. Still as this variability was mainly due to extraneous causes, no weighting of the percentages was made in the

statistical treatment. By dividing the variance into its components, a t-test was made which takes into consideration the mean extraneous variability.

(5) By repeating the test in some experimental stocks, it was discovered, that, in spite of the isogenisation work carried out, some selective factors were in action which had to be considered in the evaluation of the results.

(6) About $^3/_4$ of the irradiated third chromosomes were affected in their viability. The subvitals were at least 3,5 times as frequent as the lethals. With higher detrimental effect the frequency of the subvitals decreased.

(7) Though no conclusive answer is possible to the problem of the dominance relations of the subvitals, it is clear that dominant mutations are very frequent among the subvital mutations. Of the 4 mutations which showed significant partial dominance of the detrimental effect, the less the degree of dominance the more detrimental the mutation was.

Literature cited

BEADLE, G. W. 1951. Chemical genetics. — DUNN's Genetics in the 20th Century, pp. 221—239. The MacMillan Company, New York.

BERG, R. L. 1937 a. The relative frequency of mutations in different chromosomes of *Drosophila melanogaster*. I. Lethal mutations. — Genetics 22, 225—240.

— 1937 b. The relative frequency of mutations in different chromosomes of *Drosophila melanogaster*. II. Sterility mutations. — Genetics 22, 241—248.

COCHRAN, W. G. 1943. Analysis of variance for percentages based on unequal numbers. — J. Am. Stat. Ass. 38, 287—301.

DOBZHANSKY, TH., HOLZ, A. M. and SPASSKY, B. 1942. Genetics of natural populations VIII. Concealed variability in the second and the fourth chromosomes of *Drosophila pseudoobscura* and its bearing on the problem of heterosis. — Genetics, 27, 463—490.

DOBZHANSKY, TH. and SPASSKY, B. 1944. Genetics of natural populations XI. Manifestation of genetic variants in *Drosophila pseudoobscura* in different environments. — Genetics 29, 270—290.

— and — 1953. Genetics of natural populations XXI. Concealed variability in two sympatric species of *Drosophila*. — Genetics 38, 471—484.

— and — 1954. Genetics of natural populations XXII. A comparison of the concealed variability in *Drosophila prosaltans* with that in other species. — Genetics 39, 472—487.

GOWEN, J. W. 1952. Hybrid vigor in *Drosophila*. — Heterosis, pp. 474—493. Iowa State College Press, Iowa.

GUSTAFSSON, Å. 1946. The effect of heterozygosity on variability and vigour. — Hereditas XXXII, 263—286.

GUSTAFSSON, Å. 1947. The advantageous effect of deleterious mutations. — Hereditas XXXIII, 573—575.

— 1951. Mutation, environment and evolution. — Cold Spring Harbor Symposia on Quantitative Biology, 16, 263—281.

KÄFER, E. 1953. Vitalitätsmutationen, ausgelöst durch Röntgenstrahlen bei *Drosophila melanogaster*. — Zschr. ind. Abst.- u. Vererb. 84, 508—535.

KAUFMANN, B. P. and DEMEREC, M. 1942. Utilization of sperm by the female *Drosophila melanogaster*. — Amer. Nat. 76, 445—469.

KERKIS, J. 1938. Study of the frequency of lethal and detrimental mutations in *Drosophila*. — Izv. Akad. Nauk. SSSR (Otd. mat. -est., Ser. biol.), 75—96.

LÜNING, K. G. 1952. X-ray induced dominant lethals in different stages of spermatogenesis in *Drosophila*. — Hereditas XXXVIII, 91—107.

MASING, R. A. 1938. Increased viability of heterozygotes for a lethal in *Drosophila melanogaster*. — C. rend. (Dokl.) Acad. Sc. URSS. 20, 173—176.

— 1939. Analysis of vitality of flies of *Drosophila melanogaster* heterozygous for lethals arisen in nature. — C. rend. (Dokl.) Acad. Sc. URSS. 25, 64—67.

MEYER, H. U., EDMONDSON, M., ALTENBURG, L. and MULLER, H. J. 1949. Studies on mutations induced by ultraviolet in the polar cap of *Drosophila*. — Genetics 35, 123—124.

MULLER, H. J. 1928. The problem of genetic modification. — Verh. V. Internat. Kongr. Vererb., Suppl. I., Zschr. ind. Abst.- u. Vererb., 234—260.

— 1934. Radiation genetics. — Verh. 4. internat. Kongr. Radiol. (Zürich) 2, 100—102.

— 1936. Construction of homozygous stocks. — DIS 6, 7.

— 1943. Mutation rate dependent on the size of the X chromosome. — Genetics 28, 83.

— 1950. Our load of mutations. — Am. J. Human. Genet. 2, 111—176.

PAVAN, C., CORDEIRO, A. R., DOBZHANSKY, TH., DOBZHANSKY, N., MALOGOLOWSGIN, C., SPASSKY, R., WEDEL, M. 1951. Concealed genic variability in Brazilian populations of *Drosophila willistoni*. — Genetics 36, 13—30.

STERN, C., CARSON, G., KINST, M., NOVITSKI, E. and UPHOFF, D. 1952. The viability of heterozygotes for lethals. — Genetics 37, 413—449.

STRAUS, F. S. 1942. The genetic mechanism of heterosis as demonstrated by egg production in *Drosophila melanogaster*. — A Thesis Submitted to the Graduate College for the Degree of Doctor of Philosophy. Iowa State College. 86 p.

TIMOFÉEFF-RESSOVSKY, N. W. 1934. Auslösung von Vitalitätsmutationen durch Röntgenbestrahlung bei *Drosophila melanogaster*. — Strahlentherapie 51, 658—663.

— 1935. Auslösung von Vitalitätsmutationen durch Röntgenbestrahlung bei *Drosophila melanogaster*. — Nachr. Ges. Wiss. Göttingen (Math.-phys. Kl. Biol.) N. F., 1, 163—180.

WALLACE, B. 1950. Autosomal lethals in experimental populations of *Drosophila melanogaster*. — Evolution 4, 172—174.

WALLACE, B. and MADDEN, C. 1953. The frequency of sub- and supervitals in experimental populations of *Drosophila melanogaster*. — Genetics 38, 456—470.

THE MECHANISM OF CHIASMA FORMATION AND CROSSING OVER

A NEW HYPOTHESIS

By *FOLKE FAGERLIND*

INSTITUTE OF MORPHOLOGICAL BOTANY, UNIVERSITY OF STOCKHOLM (STOCKHOLMS HÖGSKOLA), STOCKHOLM

IT is possible that the zygotene chromosomes are bilateral structures. Already at this early point of time their splitting into chromatids may have started.

A bilateral structure of the chromosomes opens other possibilities for the syndesis than those we reckoned with earlier. A pair of homologous chromosomes are named A and B, their sides A_d (the »dorsal» side of A) and A_v (the »ventral» side of A), B_d and B_v, respectively. Hence, the following syndesis possibilities are present:

$$A_v—B_d,\ A_d—B_v,\ A_v—B_v,\ A_d—B_d.$$

The syndesis between two homologous chromosomes starts at one single point or simultaneously at several separate points. These may be called the *primary syndesis points*. From each of these the process continues in a zipper-like manner in both the possible directions. Thus, between two adjacent primary syndesis points there are two syndesis frontiers approaching each other.

The two sides which have established contact with each other at one of the primary syndesis points, are called the ventral side of A and the dorsal side of B. We may speak of v/d-syndesis if we find the same type of syndesis — $A_v—B_d$ (the ventral side of A has established contact with the dorsal side of B) — also at an adjacent primary syndesis point. We may speak of d/v-syndesis if the dorsal side of A has established contact with the ventral side of B at the point last mentioned. The two remaining possibilities are called d/d- and v/v-syndesis, respectively.

There are no obstacles at all to the meeting of the two syndesis frontiers in the simpler cases of v/d-syndesis. In other cases interesting complications are the result (*cf.* Fig. 1).

In the case of d/v-syndesis the approaching of the two syndesis

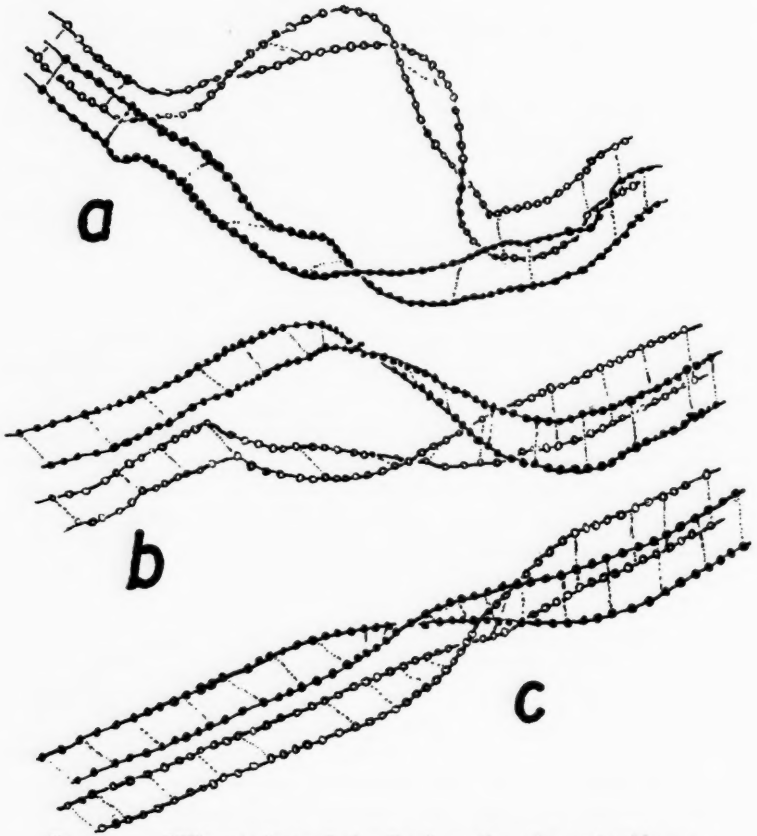


Fig. 1. *a*—*c*. Different phases during the formation of a proto-chiasma.

frontiers produces torsions in the interfrontal section. According to the different causes of the d/v-syndesis, the two components of the geminus in the said section are coiled in one and the same direction or in directions opposite to each other. If the stress conditions finally become equal in both the chromosomes the resulting picture will be quite regular. In the case in which the chromosomes are coiled in one and the same direction the torsion angle will be 90° in each of the mates just when the frontiers meet. Hence, the syndesis planes before and after the collision point will be perpendicular to each other. For the same reason each of two non-sister chromatids in a mutually diagonal

position to each other are found in one and the same quadrant before as well as after the said point. The two remaining chromatids — they are also non-sister chromatids in a mutually diagonal position — change quadrants at the collision point. Because of the pairing of the homologous points during the process of syndesis the two quadrant-changing chromatids will cross each other at a point where »homologous interparticles» are pressed against each other.

The fragmentations, inversions, translocations, *etc.*, of the chromosomes support the opinion that the essential particles of the chromosomes are held together by forces which are very easily overpowered. The conception of the chromosome as a pile of essential particles intermingled in a highly viscous filling mass and held together by this or merely by simple interparticulate forces of another kind seems plausible.

If two stretched, highly viscous threads — for instance, threads of concentrated Canada balsam — are brought together in such a way as to intimate the quadrant-changing chromatids, the immediate result is a melting together of the threads into a central cross. The cross, however, is a quite unstable structure. According to the way in which we manoeuvre the arms of the cross, the original pair of threads is reconstructed or a new one created. The new construction is the consequence of a »crossing over» between the original threads at their fusion point. The physical basis of the different processes is easily understood.

On the assumptions postulated above, the quadrant-changing chromatids will also form a central unstable cross. On the same assumptions, the cross will be replaced by a pair of threads which are the same as the original ones or are new structures created by crossing over between the original chromatids at the fusion point.

In the case first mentioned the chromosome sections concerned will move apart in the diplotene and diakinesis nucleus; in the second case chromosome sections will repel each other on each side of the collision point but they will be held together themselves as a consequence of the executed recombination.

If the above ideas are correct we have to distinguish between two different types of chiasmata. The first type, the unstable crosses, I term *proto-* or *fusion-chiasmata.* These are eliminated without any complications or, by means of crossing over, are transformed into the second type, *viz.* into *meta-* or *crossover-chiasmata.*

The hypothesis now put forward postulates the presence of more than one primary syndesis point in one and the same geminus. How-

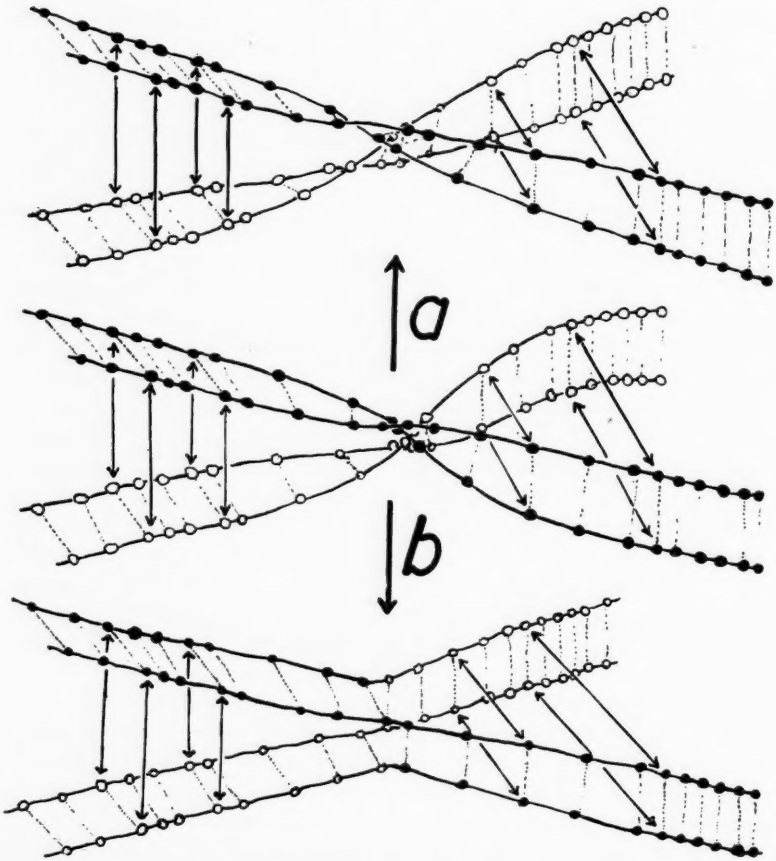


Fig. 2. *a*. The proto-chiasma elimination without any complications. — *b*. The transformation of a proto-chiasma into a meta-chiasma by means of crossing over. The slender arrows illustrate the repulsion forces during diplotene.

ever, it is not necessary for these points to be directly detectable in the slides. It is possible that the leptotene chromosomes are coiled along their own longitudinal axis. There are facts to support the view that the splitting plane of the chromosomes is slightly spiralized. Thus, when we speak of a complete syndesis we may possibly only have an illusory impression. The process is perhaps only completed at a few points; the interspatial parts may not complete their syndesis until more or less time-demanding torsions have been executed.

The following theses have been proved or have been almost proved to be true:

(1) The centromere part of the geminus is pre-reduced.

(2) The chromosome parts repelling each other during diplotene and later are formed by sister chromatids.

(3) In crossing over the two participating chromatids are broken and reunited at homologous points.

(4) At each crossing over point only two of the four chromatids participate. These are non-sister chromatids.

(5) The crossing over points and the chiasmata show interference.

(6) Chiasmata exist also in cases where crossing over is lacking.

(7) If the chiasmata frequency is altered by some extra factors the crossing over frequency is altered in the same direction.

(8) The planes on both sides of a given chiasma in which the chromosomes repel each other during diakinesis are perpendicular to each other.

(9) In double crossing over 2 as well as 3 and 4 threads participate. — These different cases are the consequence of the different syndesis types.

(10) In double chiasmata the types »free, continuous, chromosome-locked and chromatid-locked» (*cf.* SAX, 1936; HUSKINS and NEWCOMBE, 1941) and still more complicated cases are all found. — These different types are the consequence of the different syndesis types and the different fates of the proto-chiasmata.

(11) The average distance between the two chiasmata in the »free» type is shorter than in the other types mentioned above.

All these eleven points are entirely in agreement with the new ideas. Hence, the new hypothesis is a trustworthy one. If new ideas (FAGERLIND, 1955) about the spiralization cycle of the chromosomes are also added, several cytological problems — also as puzzling appearances as those observed during the development cycle of ring-chromosomes and of the attached X-chromosomes in *Drosophila* — are easily understood.

A paper including short references to the different crossing over and chiasma hypotheses — from the precursors of GRANATA and JANSSENS to SCHWARTZ (1954) — which comprises a critical examination of these and a detailed analysis of the new hypothesis and its consequences is now in preparation.

Literature cited

FAGERLIND, F. 1955. The spiralization cycle of the chromosomes. A new hypothesis. — (In manuscript.)

HUSKINS, C. L. and NEWCOMBE, H. B. 1941. An analysis of chiasma pairs showing chromatid interference in *Trillium ercetum* L. — Genetics 26: 101—127.

SAX, K. 1936. Chromosome coiling in relation to meiosis and crossing over. — Genetics 21: 324—338.

SCHWARTZ, D. 1954. Studies on the mechanism of crossing over. — Genetics 39: 692—700.

BRIEF REPORTS

A. JOSEFSSON: Tetraploid turnips a progress in Swedish root crop breeding.

At the Swedish Seed Association, tetraploids of most field crops are produced by colchicine treatment. Most successful among these new artificially induced autotetraploids are those which have a rather low number of chromosomes, are cross fertilizable and where the vegetative parts are used. Thus, root crops have been a good object for chromosome doubling experiments. This is especially the case with turnips. The diploid chromosome number is $2n=20$. It is a cross fertilizer and the crop is grown to obtain the roots. The tetraploids often have a high vegetative production. The dry matter content, however, is somewhat low, but the yield of dry matter is high. The artificial autotetraploids have, as a rule, a decreased fertility, but the yield of seed is not of the same importance to the root crops as to other fårm crops.

Doubling the chromosome number of some varieties of turnips was started by Dr. ALBERT LEVAN about fifteen years ago. In the spring of 1946 some tetraploid seeds of the three varieties Bortfelder, Yellow Tankard and Östersundom were sown for the first time in a comparative test with diploid and tetraploid turnips. In 1951 a seed stock of tetraploid turnips was handed over to the General Swedish Seed Company for propagation and in 1953 this stock was marketed under the name of »Svalöf's tetraploid Sirius turnip».

The breeding and testing of the tetraploid turnips have been carried out in close co-operation between the main station and the different branch- and sub-stations of the Seed Association in districts where turnips are normally grown. A special breeding program of the tetraploids was started at the Jämtland branch station as well as the Svalöf station. Crosses have been made at two different branch stations between all tetraploid strains available. The progenies of these »mass crosses» have been tested in field trials and have been shown to give a very high yield of roots and dry matter. Some crosses between tetraploid strains of Bortfelder, Yellow Tankard and Östersundom have shown excellent results. The tetraploid Sirius is an F_1 variety derived from crosses between some strains of these three different types.

In breeding tetraploid turnips two methods are used. One of them tends to produce tetraploid strains quite similar to the diploid mother strains as to type and colour of root. The other method, as mentioned above, is to produce a very heterozygous stock. It is possible to get four different alleles at each locus in one and the same plant. Thus, theoretically, there is a greater probability that a tetraploid will be heterozygous at any particular locus than a diploid. Thus, tetraploid plants can utilize the effect of heterozygosity better than diploids. In the trials described below, extending over 7 years (1948—1954) at 7 different stations, tetraploid strains have been compared with the corresponding diploid mother strain; and »mass crossed» material, especially the variety Sirius, is compared with the highest yielding of the constituent diploid strains. All the strains have not been tested each year owing to lack of seed.

All the tetraploids had a good and well-formed root but the dry matter content of the root was somewhat lower in tetraploids than in diploids. The dry

TABLE 1. *Results of field trials with tetraploid turnips at the Swedish Seed Association 1948—1954.*

Variety	Number of trials	No. of plants per ha	Yield of roots		Dry matter			Yield of tops		
			dt/ha	rel.	%	dt/ha	rel.	no. trials	dt/ha	rel.
Svalöf's Bortfelder, 2*n*	38	77345	489	100	9,9	48,5	100	28	242	100
» » , 4*n*	38	75800	562	115	9,1	51,0	105	28	209	86
» Yellow Tankard, 2*n*	27	78785	454	100	10,2	46,3	100	20	255	100
» Yellow Tankard, 4*n*	27	79410	621	137	9,1	56,4	122	20	180	71
» Östersundom, 2*n*	32	76420	602	100	9,0	54,1	100	24	193	100
» Östersundom, 4*n*	32	74960	657	109	8,6	56,8	105	24	172	89
» Bortfelder, 2*n*	33	81420	493	100	10,0	49,5	100	27	237	100
» Sirius, 4*n*	33	75480	690	140	8,7	60,3	122	27	204	86
» Östersundom, 2*n*	32	76330	607	100	9,1	55,1	100	24	186	100
» Sirius, 4*n*	32	75620	700	115	8,7	61,0	111	24	192	103

The difference in dry matter in the last comparison corresponds to $t = 3{,}20^{**}$; $0{,}01 > P > 0{,}001$.

matter yield, however, was higher and the tetraploids seemed to have a more rapid development than the diploids. The tetraploids also seemed to maintain their superiority under unfavourable as well as under favourable conditions.

As shown in Table 1, tetraploid Bortfelder has been compared with diploid in 38 trials. The tetraploid has usually given 15 per cent higher yield of roots and 5 per cent higher yield of dry matter than the diploid. The dry matter content, however, was 0,8 per cent lower in the tetraploid crop. The tetraploid strain of Yellow Tankard tested is very high yielding. The root yield was 37 per cent and the yield of dry matter was 22 per cent higher in this tetraploid than in the corresponding diploid material. The tetraploid strain of Östersundom has not given so good results. The figures were 9 and 5 per cent, respectively. In some trials this tetraploid has yielded less than the mother strain. The yield of tetraploid Östersundom fluctuated more than that of the other tetraploids.

Sirius has been tested in 33 trials and has outyielded Bortfelder in dry matter yield by 22 per cent and Östersundom by 11 per cent. The content of dry matter was only 8,7 per cent while the diploid standards had 10,0 and 9,1, respectively. The roots of Sirius turnips are medium long and are big and well-formed. The outside colour is very variable and the flesh of most roots is yellow.

Root crops are mainly grown for carbohydrate production but the other components of dry matter are of course also of some importance. Some data

TABLE 2. *The quality of dry matter in roots.*

	Sirius, 4*n*	Bortfelder, 2*n*	Östersundom, 2*n*
Crude protein (N × 6,25)	11,0	13,5	14,1
Crude fiber	13,5	13,1	13,1
Ash	6,9	8,1	9,6
N-free extract	66,6	63,3	61,2

on the composition of dry matter are given in Table 2. The content of crude protein and that of ash was lower in tetraploids than in diploids. The content of nitrogen-free extract (carbohydrate a. o.) was, however, higher.

The yield of tops was lower in the tetraploids than in the diploids, *e. g.*, the yield of Sirius was only 86 per cent of that of the diploid strain of Bortfelder.

Seed of tetraploid turnips are larger than seed of diploids, the thousand grain weight is about 3,8 g and 2,4 g, respectively. The yield of seed has not been carefully investigated. At Svalöf and the branch stations separate field plots have been grown for seed production of breeding material. As a rule, those with tetraploid turnips have yielded rather well. Three rather large propagation fields with tetraploid turnips (Sirius) have been grown for seed production. Two of them yielded normally, but the third yielded less due to weak and uneven stand and development of the plants. The seed quality of Sirius has been perfect.

There are no definite observations on resistance to club root (*Plasmodiophora brassicae*) and to bacterial disease (*Pseudomonas erwiniae*). It was observed, however, that the tetraploids tend to be a little more resistant to club root but less resistant to bacterial disease than the corresponding diploid material.

The tetraploid Sirius turnip doubtless marks a progress in Swedish root crop breeding. It is more than 10 per cent higher yielding than the best of the diploid strains under the various conditions in different parts of our country. This is an outstanding example of what can be done by the »polyploidy breeding method». The breeding of turnips by chromosome doubling has been intensified and most of the diploid strains and »families» have been colchicine treated and tetraploid populations established. As many genes and alleles as available may be brought over to the tetraploid populations and the breeding of the tetraploids carried out by the two methods outlined earlier. We want higher dry matter content, especially protein content, and better resistance to disease. The most important goal, of course, is a high and safe yield of dry matter. There seems to be a good possibility of obtaining this by intensive breeding of tetraploid turnips.

A. NYGREN: Polyploids in *Melandrium* produced by nitrous oxide.

Quite recently, ÖSTERGREN (1954) has published a paper on the production of polyploids in *Crepis capillaris* by N_2O treatment. During a short visit to

TABLE 1. *Results of nitrous oxide (N_2O) treatment in Melandrium.*

Nos.	Combination	Treatment	Hours	No. of plants	Chromosome Nos. ± 24	± 36	± 48	± 72	Ch.
26—54	*M. divaricatum* (139) × *M. album*	2 atm.'s	16	12			4	7	50,72
27—54	» (139) × *M. divaricatum*	5 »	4	1	1				
32—54	» (140) × *M. album*	5 »	12	5			5		
35—54	» (140) × » (149)	10 »	4	5	5				
36—54	» (140) × *M. album*	5 »	16	3		2			
38—54	» (140) × » (149)	10 »	4	5	3		2		
39—54	» (140) × *M. Boissieri*	5 »	16	4		4			
40—54	» (140) × *M. album*	5 »	6	3		1	2		
43—54	*M. album* (149) × *M. album*	5 »	5	1			1		
48—54	» (151) × »	5 »	16	5		2			
50—54	» (151) × *M. Boissieri*	10 »	5	20	18		1		
53—54	» (151) × *M. macrocarpum*	5 »	16	2	2				
57—54	*M. macrocarpum* × *M. macrocarpum*	2 »	16	5	3				
62—54	*M. album* × *M. album*	5 »	5	10	1		7		
63—54	» × »	5 »	4	10			3		
64—54	» × »	5 »	7	10			10		
72—54	» × »	5 »	8	4			3		
75—54	» × »	5 »	6	9			4		
76—54	» × *M. macrocarpum*	5 »	6	9	4				
77—54	» × *M. album*	5 »	4	10			8		
78—54	» × » (149)	10 »	4	10	6				
80—54	*M. divaricatum* (139) × *M. album*	5 »	6	3		2	1		
83—54	*M. Boissieri* × *M. macrocarpum*	5 »	6	4	2		1		24,47
84—54	» × *M. album* (149)	10 »	4	10	8				24,48
85—54	» × »	5 »	16	1		1			
91—54	» × »	5 »	16	6			4		
94—54	*M. album*	4 »	4	10			3		
				177	53	12	59	7	3

TABLE 2. *Number of polyploids and diploids after different treatments with N_2O.*

Atm.'s	No. of treatments	No. of counted plants	Polyploids	Diploids
2	2	15	12	3
4	1	3	3	—
5	19	72	62	10
10	5	44	4	40
Sum —	27	134	81	53

Lund in the spring of 1953 the present author had the privilege of being introduced to the work carried on with nitrous oxide by Dr. ÖSTERGREN. As a result of this visit an attempt has been made to produce polyploid *Melandrium* in the same way as ÖSTERGREN produced polyploid *Crepis capillaris*.

The plants were treated in vessels of two different sizes made by old gas tubes holding about 8 and 36 l, respectively. The tubes were cut of at their upper end, made even and furnished with covers which could be connected to the reduction valve of the nitrous oxide cylinder. The two vessels allowed the treatment of material planted in pots of different sizes. Up to six 15 cm high plants could be treated at the same time in the smaller container, while the big tube took three 50 cm high specimens. Two different species of *Melandrium* were tested, *M. angustiflorum*, a hermaphroditic tetraploid, which is related to the Greenlandic *M. affine*, and the dioecious *M. album*. As early as 1950 the author had made hybrids between *M. angustiflorum* and dioecious *M. rubrum*. In 1953 nitrous oxide treatment was used in order to produce amphidiploids directly. The dioecious *M. album* was tested together with its South European ecotype *M. divaricatum* (=*M. macrocarpum*) as well as the closely related species. *M. Boissieri*. The first division in the zygote should be affected by the nitrous oxide. This division occurs in *M. album* about 12—14 hours after pollination at a temperature of 35° C (DEVINE, 1950). As we had no constant rooms at our disposal during the time the experiments were going on, the treatment in each particular case was started between 18 and 24 hours after pollination. Before pollination the plants were kept in a greenhouse with a temperature varying between 20 and 25° C.

All attempts to obtain amphidiploids directly between *M. angustiflorum* and *M. rubrum* failed in 1953, but on the other hand the experiments with *M. album* and its allies were successful. All plants belonging to this and related species are dioecious, and crosses were therefore made either between different biotypes of the same species or between biotypes of related species. The nitrous oxide treatment varied between four and 48 hours at pressures between two and ten atmospheres. All plants treated for a longer time than 16 hours set no seeds. The results are to be found in Table 1. It is striking that only three chimaeras have been found in 81 polyploids produced. Only seven of the 78 real polyploids have aneuploid numbers; thus, there are two with $2n=37$, two with $2n=47$ and three with $2n=50$. The results found are condensed in Table 2. It is important to note that treatment in five atmospheres has given not less than 86,1 % polyploids. Thus, there will be no difficulties in the future to produce polyploid *Melandrium album* with this method. ÖSTERGREN obtained

three triploids of *Crepis capillaris* in his experiments (*l. c.*, p. 59). In the present case 12 triploids and seven hexaploids were produced. ÖSTERGREN supposes (p. 61) that the triploids originate according to a particular mechanism causing »multipolar chromosome separation». If the telophase groups of some of these spindles fuse, triploids as well as aneuploids may be formed. The theory gives a very probable explanation of the origin of triploids, and the finding of hexaploids in the experiments with *Melandrium album* only strengthens its correctness. All hexaploids were obtained in the same experiment during which a pressure of only two atmospheres was used for 16 hours. It is possible that low pressure for a long period has allowed two consecutive divisions of the chromosomes in the zygote to occur without cell-wall formation.

Literature cited

DEVINE VERONA. 1950. Embryogeny of *Lychnis alba*. — Am. Journ. of Bot., 37: 197—207.

ÖSTERGREN, G. 1954. Polyploids and aneuploids of *Crepis capillaris* produced by treatment with nitrous oxide. — Genetica XXVII: 54—64.